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Collection management and study of microscope slides: Storage, profiling, deterioration, restoration procedures, and general recommendations

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Abstract

A wide range of aspects concerning microscope slides, their preparation, long-time storage, curatorial measures in collections, deterioration, restoration, and study is summarized based on our own data and by analyzing more than 600 references from the 19th century until 2016, 15 patents, and about 100 Materials Safety Data Sheets. Information from systematic zoology, conservation sciences, chemistry, forensic sciences, pathology, paleopathology, applied sciences like food industry, and most recent advances in digital imaging are put together in order to obtain a better understanding of which and possibly why mounting media and coverslip seals deteriorate, how slides can be salvaged, which studies may be necessary to identify a range of ideal mounting media, and how microscope studies can benefit from improvements in developmental biology and related fields. We also elaborate on confusing usage of concepts like that of maceration and of clearing.

The chemical ingredients of a range of mounting media and coverslip seals are identified as much as possible from published data, but this information suffers in so far as the composition of a medium is often proprietary of the manufacturer and may vary over time. Advantages, disadvantages, and signs of deterioration are documented extensively for these media both from references and from our own observations. It turns out that many media degrade within a few years, or decades at the latest, except Canada balsam with a documented life-time of 150 years, Euparal with a documented life-time of 50 years, and glycerol-paraffin mounts sealed with Glyceel, which represents almost the only non-deteriorating and easily reversible mount. Deterioration reveals itself as a yellowing in natural resins and as cracking, crystallization, shrinkage on drying or possibly on loss of a plasticizer, detachment of the coverslip, segregation of the ingredients in synthetic polymers, as well as continued maceration of a specimen to a degree that the specimen virtually disappears. Confusingly, decay does not always appear equally within a collection of slides mounted at the same time in the same medium. The reasons for the deteriorative processes have been discussed but are controversial especially for gum-chloral media. Comparing data from conservation sciences, chemical handbooks, and documented ingredients, we discuss here how far chemical and physical deterioration probably are inherent to many media and are caused by the chemical and physical properties of their components and by chemicals dragged along from previous preparation steps like fixation, chemical maceration, and physical clearing. Some recipes even contain a macerating

agent, which proceeds with its destructive work. We provide permeability data for oxygen and water vapor of several polymers contained in mounting media and coverslip seals. Calculation of the penetration rate of moisture in one example reveals that water molecules reach a specimen within a few days up to about a month; this lays to rest extensive discussions about the permanent protection of a mounted specimen by a mounting medium and a coverslip seal.

Based on the ever growing evidence of the unsuitable composition and application of many, and possibly almost all, mounting media, we strongly encourage changing the perspective on microscope slides from immediate usability and convenience of preparation towards durability and reversibility, concepts taken from conservation sciences. Such a change has already been suggested by Upton (1993) more than 20 years ago for gum-chloral media, but these media are still encouraged nowadays by scientists. Without a new perspective, taxonomic biology will certainly lose a large amount of its specimen basis for its research within the next few decades. Modern non-invasive techniques like Raman spectroscopy may help to identify mounting media and coverslip seals on a given slide as well as to understand ageing of the media. An outlook is given on potential future studies.

In order to improve the situation of existing collections of microscope slides, we transfer concepts as per the Smithsonian Collections Standards and Profiling System, developed for insect collections more than 25 years ago, to collections of slides. We describe historical and current properties and usage of glass slides, coverslips, labels, and adhesives under conservational aspects. In addition, we summarize and argue from published and our own experimental information about restorative procedures, including re-hydration of dried-up specimens previously mounted in a fluid medium. Alternatives to microscope slides are considered. We also extract practical suggestions from the literature concerning microscope equipment, cleaning of optical surfaces, health risks of immersion oil, and recent improvements of temporary observation media especially in connection with new developments in digital software.

Key words: mounting medium, coverslip seal, fixation, maceration, clearing, immersion oil, re-hydration, permeability

1. Introduction

Natural history museums house large amounts of specimens in wet collections, as dry mounts, and on microscope slides. Especially small invertebrate specimens like worms, insects, crustaceans, and mites are mounted as whole mounts on microscope slides and studied by transmitted light microscopy. The slides include also body parts of medium-sized animals and histological sections as well as both type and voucher material. Quite frequently, researchers in taxonomy want the inner organs of the specimens to be removed in order to study just the cuticular exoskeleton. The consequence of this requirement is disastrous: specimens are not only macerated and not necessarily sufficiently washed before permanent mounting, but specimens are often even mounted in a mounting medium that macerates specimens and continues to do so over time. In addition, media deteriorate, crystals in the medium and on or close to the specimen, cracks, and cavities in the media develop commonly, the components of the media may segregate, the coverslip may detach (references in Tabs 6, 7). Some of the most problematic media include Aquatex®, polyvinyl lactophenol, Fluoromount G™, gum-chloral based media like Berlese's, Faure's, and Hoyer's mounting medium, and Permount™ (Tab. 7; Hooper 1970; Upton 1993; Koomen & Vaupel Klein 1995; Amrine & Manson 1996; Brown 1997; Lillo *et al.* 2010; Jacinavicius *et al.* 2013; Neuhaus 2013, 2017; Neuhaus & Sørensen 2013; Neuhaus *et al.* 2014; Neuhaus & Kegel 2015). The serious problems with the above-mentioned mounting media have been widely ignored for decades (references in Tabs 6, 7; for summary about gum-chloral media see Upton 1993), and these media are still recommended with sometimes bizarre arguments (Tab. 7) until today (Walter & Krantz 2009; Criado-Fornelio *et al.* 2014) despite the enormous body of evidence about their non-permanence (references in Tabs. 6, 7). Some curators of large museum collections are more aware of the deterioration processes, because they have direct access to an enormous amount of microscope slides from different time periods and mounted with different mounting media (Upton 1993; Brown 1997; Neuhaus 2013; this paper), whereas scientists at universities often seem to focus mainly on how to get satisfying slides in a short time and with as little effort as possible. Several taxonomically important collections have already been lost or will be lost in the foreseeable future if no measures will be undertaken to salvage these collections (Schauff 1985; Sterrenburg 1990; Upton 1993; Brown 1997; Jersabek 2005; Jersabek *et al.* 2010; Lillo *et al.* 2010; Neuhaus pers. obs.).

In the area in which the senior author does his own research, various mounting media like CMC-10, Fluoromount G™, glycerol, Hoyer's mounting medium, and Permount™ are documented in the literature for slides of the meiobenthic taxon Kinorhyncha, and problems like too intense maceration of specimens have been recognized for a long time for some media (Higgins 1960, 1961, 1964, 1977, 1982, 1983, 1988; Westheide &

Purschke 1988; Sørensen & Pardos 2008; Neuhaus 2013). It is only recently, that additional severe problems with several of the above-mentioned mounting media have been mentioned such as the formation of cavities and crystals and disintegration of the medium (Neuhaus 2013, 2017; Neuhaus & Sørensen 2013; Neuhaus *et al.* 2014; Neuhaus & Kegel 2015). Unfortunately, little effort has been made to overcome the problems such as by reducing the amount of chloral hydrate in Hoyer's mounting medium by about 37% and sealing the coverslip with Murrayite or an epoxy paint (Higgins 1977, 1982, 1983, 1988). In a few cases, specimens have been re-mounted as glycerol-paraffin slides (Neuhaus & Kegel 2015; Neuhaus 2017). Procedures for re-mounting slides are scarce and offer different solutions for different taxa and different types of slides (Moore 1979; Fain 1980; Garner & Horie 1984; Brunner & Blueford 1986; Brown & Boise 2005, 2006; Gunter & Brown 2005; Woessner 2005; Allington-Jones 2008; Jacinavicius *et al.* 2013; Valentine-Baars & Kerbey 2014).

Museum collections with their broad range of microscope slides from different sources suffer widely from a lack of information about processing of the specimens, the mounting medium, and the coverslip seal used for a specific slide. Statements about the longevity of mounting media and seals are difficult to base on dated and documented specimens (but see Cushing 2011). Often, experience of newly introduced mounting media is limited to a couple of months or years (Tabs 6, 7, 9; Gater 1929; Gibbins 1930; Kirkpatrick & Lendrum 1939, 1941; Brown 1997; Criado-Fornelio *et al.* 2014). Methods for non-destructive identification of such substances from materials science like X-ray fluorescence analysis and infrared spectroscopy have been applied very rarely (Garner & Horie 1984; Valentine-Baars & Kerbey 2014). Also, the properties of mounting media and coverslip seals have rarely been tested by experiments (Allington & Sherlock 2007a, 2007b). This unsatisfactory situation makes restoration of slides a time-consuming game of trial and error with an uncertain outcome.

The problem of deteriorating museum items, especially related to plastics, adhesives, consolidants, and varnishes is not unique to natural history collections (Baker 1995) but happens in museums of fine arts as well (Blackshaw & Ward 1982; Horie 1983, 2011; Jackson 1982; Koob 1982; Witte 1983; Blank 1990; Robson 1992; Griffith 1996; Keneghan 1996; Mills & White 1999; Oosten 2002a, 2002b; Shashoua 2008). Here, the preventive (= passive) approach is to create a storage environment minimizing deterioration processes and to avoid substances that have been proven to become instable over a limited time frame; the interventive (= active) approach is to apply conservation techniques that are reversible if ever possible, and well documented (Horie 1983, 2011; Mills & White 1999; Michalski 2002; Davison 2003; Shashoua 2008). The Conservation and Preservation of Natural Science Collections Project of the National Institute for the Conservation of Cultural Property, USA suggests *inter alia* a technology transfer from related disciplines like chemistry, physics, materials science, and conservation science to natural history collections (Duckworth *et al.* 1993). Such efforts have been seen in amber collections, and specific studies have been designed to understand better the effect of environmental parameters on the deterioration of amber (Thickett *et al.* 1995; Pastorelli 2011; Pastorelli *et al.* 2011, 2012, 2013; Bisulca *et al.* 2012). These observations on amber are quite indicative for the storage conditions of microscope slides mounted in natural plant resins like Canada balsam, which has been used as mounting medium for more than 180 years now (Bracegirdle 1978, p. 88).

The Collections Committee of the Department of Entomology at the National Museum of Natural History, USA, defined the first quantitative assessment of a natural history collection in terms of its curation status including the conservation condition of the material in order to allow priority setting for the management of the collection in times of limited personal and financial resources (McGinley 1989, 1992). This Smithsonian Standards and Profiling System also provides data ante and post curation activity, so project-based activities can be easily documented for funding bodies. The system has been subsequently adapted for wet and dry vertebrate collections (Williams *et al.* 1996), paleontological collections (Adrain *et al.* 2006), and wet collections (Neuhaus *et al.* 2012). It was also suggested for microscope slides by Brown (1997, p. 11) and inspired the senior author to develop a form sheet in order to assess slide collections (Tab. 2).

It does not seem to be self-evident that animal specimens must be fixed properly for morphological studies in order to keep the specimens (hopefully) for centuries. Again and again, ethanol-preserved specimens are offered for deposition in museum collections. It seems that formaldehyde-fixed specimens are still more suitable for morphological studies and last longer than ethanol-fixed specimens (e.g., Steedman 1976a; Kiernan 1999). Also, chemicals used for maceration of internal organs (Lambers 1950; Richards 1964; Bink 1979; Böck 1989; Upton 1993; Brown 1997; Kiernan 2015; Brown & Boise 2005, 2006) may not have been removed entirely and may react with the mounting medium. Clearing agents may pose a similar problem.

The situation described above has been the incentive for the senior author to review the biological literature for descriptions of problems and solutions and to extend the search to other fields like conservation science in order to gain a different perspective on how to deal with microscope slides. Many aspects of the current paper are based on anecdotal reports in the literature. A systematic approach to understand microscope slides in all their aspects is missing widely, but few papers stand out (Upton 1993; Brown 1997). Data from chemistry and applied sciences complement the information gathered. There is now an ever growing body of evidence that many mounting media and all animal taxa are impacted, the problem of the durability of microscope slides seems to be a universal problem, not a problem of individual media and taxonomic groups (Tabs 6, 7). The aim of the present paper is (1) to provide a tool for evaluating the curation status of a microscope slide collection, (2) to summarize and discuss old and new information about slide deterioration with some focus on meiofauna including results from conservation studies, from the few experimental tests on slides available, from chemistry, and from applied sciences, (3) to suggest procedures for the restoration of slides based on current knowledge, (4) to summarize general recommendations about storage, collection management, and study of slides, and (5) to stimulate discussion about and research on the preparation and restoration of microscope slides.

2. Material and methods

Both historical, contemporary, and some more unusual mounting media and coverslip seals are treated in this article as comprehensively as seems reasonable, because chances are high that even products that are no longer produced due to their health risk for humans (e.g., Aroclor®, Caedax) will exist in a museum collection. The more common and better documented media are listed in Tables 5, 6, 8, and 9, Figures 6–14, 21–26, and mostly also in the text, but media for which less detailed information could be gathered are only mentioned in the text. Properties of the media in Tables 5, 6, 8, and 9 are certainly also true for the properties of chemically related media. For Alcarin, Clearax (Göke 2000: formation of crystals after several years), Diatopan, Dirax C, Gurr's Neutral Mounting Medium, Histoclad®, Histokitt, Histomount, Mahady Micromount, Malinol, Meltmount, Mikrops, Neo-Mount®, Omnimount™ (deterioration problems known), Plexisol, Pontalite, Pro-Texx, UV-Inert, Vinylite, XAM (not available after 2007), ZRax, data is so sparse that they are not treated in detail (Skiles & Georgi 1937; Lillie *et al.* 1950, 1953; Gray & Wess 1951; Beck 1959; Meller 1985; Westheide & Purschke 1988; Koomen & Vaupel Klein 1995; Brown 1997; Göke 2000; Wiggins & Drummond 2007).

Information about the chemical composition of mounting media and coverslip seals is kept as close as possible to the original data taken from published sources. Occasionally, chemical names are carefully adapted to current use, synonyms are provided in several cases, but generally we refer to widespread popular names. No efforts are made to apply a modern chemical nomenclature. We also provide the chemical formulae of a range of mounting media, coverslip seals, and other chemicals applied for slide mounting if individually identifiable chemicals are known (Figs 6–14, 21–26); the structure of synthetic polymers is shown as composed of general repeated units (Fig. 12). The composition of mounting media (Tab. 5) and of coverslip seals (Tab. 8) has been extracted from the literature, Materials Safety Data Sheets, and patents, but probably not all ingredients are mentioned, because they are proprietary of the manufacturers. The composition of Naphrax™ and of natural resins like Canada balsam, gum arabic, gum colophony, gum sandarac, and Venetian turpentine (= Venice turpentine) is based on the analysis of original ingredients or of raw resins in the literature; the final products will contain fewer or different chemicals, mainly because of heating during manufacture of the final product and oxidation processes (Lillie *et al.* 1953, p. 58; Mills & White 1999, p. 100; Scalarone *et al.* 2002, p. 348). In Tables 5 and 8, a chemical that is liquid at 25°C is regarded as solvent, whereas the remaining chemicals are placed in other categories. One or two plates are dedicated to the chemical formula of each natural resin in order to show their complex chemical composition (Figs 6–11, 21–26). Consequently, a few chemicals appear repeatedly on different plates. The composition of one and the same product like Canada balsam seems to vary between different manufacturers. For several media we find different names and different chemical components in the literature, e.g., for Caedax, C-M Medium, CMC, CMCP, and Permount™. In these cases, it usually remains open at which time which recipe of a medium was applied. For Loctite® 363™ Impruv® Potting Compound Light Cure and Technovit® 7100, Materials Safety Data Sheets from different dates reveal a different chemical composition at a given time (Tab. 5). This general problem has been recognized early (Gray 1954, p. 651). Many substances mentioned in the article bear considerable health risks for

humans, so the reader is strongly encouraged to check MSDS and databases like GESTIS Substance Database (= Information system about hazardous substances; Available from: http://www.dguv.de/ifa/index-2.jsp) and NIOSH Pocket Guide to Chemical Hazards (Available from: http://www.cdc.gov/niosh/npg/) before experimenting and applying older recipes.

Observations and experiments from conservation science about similar or identical media as used for microscope slides are discussed, because similar data is widely missing for slides. It has to be kept in mind that such studies often refer to varnishes used on pictures. Consequently, such media possess an extremely high surface-to-volume ratio exposing a significantly larger surface of the media to environmental conditions like light, oxygen, and other gases and vapors than media of microscope slides. Also, criteria of ageing differ partly (see chapter 4.1 Mounting medium and coverslip seal: durability *versus* reversibility).

We make considerable efforts to obtain and refer to original references whenever possible and try to cover a broad range of taxa. Additional taxon-specific literature can be found in Brown (1997). We also refer to publications from the early and middle 20th century (e.g., James 1887; Behrens 1892; summary of recipes from older literature in Gatenby & Beams 1950; Gray 1954), because older museum collections certainly harbor microscope slides from that time suffering from the problems described at that time. In addition, we cite amateur and "grey" literature like newsletters of specialists in a given field, e.g., articles providing long-time experience with mounting media that is sparsely documented otherwise.

Many publications provide data on the refractive index of a mounting medium. However, few papers indicate the temperature at which the index was measured and whether the medium was measured in the dry or in the dissolved condition. Therefore, we mainly, but not exclusively, mention the refractive index (Tab. 4) if the temperature and the state are indicated in the literature.

Both historical and contemporary information about slides, coverslips, and microscope objectives is mentioned, because natural history collections house such microscope slides, and older microscopes before the era of microscopes with an infinite optical tube length are still in use at such institutions.

Specimens were photographed with a Zeiss AxioCam MRc5 and objectives Plan-Apochromat 10x/0.32, 20x/0.60 as well as Plan-Neofluar 40x/0.75 and 40x/1.30 Oil attached to a Zeiss Axioplan 2 mot. For lower magnifications, a Zeiss AxioScope A.1, Zeiss AxioCam MRc5, and objectives Plan-Neofluar 1.25x/0.035, 2.5x/0.075, and 5x/0.16 Ph1 were used. Objects and entire slides were photographed with a Canon EOS 50D, a compact-macro lens EF 50 mm/2.5, and a macro ring light Canon MR-14EX. The images were digitally improved with Corel Photo Paint V.16 and mounted with Corel Draw V.16.

List of abbreviations

comp.	compare
DIC	differential interference contrast after Nomarski
nA	numerical aperture
MSDS	Materials Safety Data Sheets
$nD^{20°C}$	refractive index at 20°C and at wave length 589 nm (= D-line of sodium)
pers. com.	personal communication
pers. obs.	personal observation
RH	relative humidity.

3. Results and discussion

3.1 Specimen processing

3.1.1 Primary fixation and preservation

Long-time preservation of specimens for light microscopy on microscope slides depends also on initial fixation. Chemical fixation or at least preservation of biological specimens is facilitated either by coagulant dehydrating preservatives such as ethanol, non-coagulant cross-linking fixatives such as formaldehyde and chloral hydrate, or compound fixatives such as alcoholic formalin (Grizzle *et al.* 2008). There are plenty of recipes for more special

purposes (Gray 1954, pp. 182–266), e.g., immature insects are often preserved in a mixture of kerosene, ethanol, glacial acetic acid, and either dioxane or a detergent like Triton X-100 or Tween (Stehr 1987). Small and delicate marine plankton is fixed in a mixture of acrolein, glutardialdehyde, and tannic acid (Veer 1982) or in 2% acidified Lugol solution (Jaspers & Carstensen 2009). Kiernan (2015, pp. 12–42) provided for different fixatives an overview of how they react with the tissues and which advantages and drawbacks they offer.

Curran & Hominick (1980) reported that temperature and exposure time during heat killing, components and temperature of fixation fluid, and processing to mounting medium affected shape, appearance of cellular and cuticular characters, and several measurements of nematodes. They also concluded that "no combination of methods produced specimens with all the characters of live nematodes, and the two species were not equally sensitive to the different treatments" (Curran & Hominick 1980, p. 463). Therefore, these authors suggested carefully documenting every step of the preparation of a specimen. Artifacts in histological sections originating at the different stages of tissue processing were described by Wallington (1979) and McInnes (2005).

Alcohols. Ethanol seems to have been used as preservative first about 1662 by Robert Boyle (Tab. 1; Reid 1994, p. 32). Ethanol is a coagulant preservative precipitating proteins by dehydration and, consequently, stopping its biological activity (Böck 1989, pp. 72–74, 80–81; Eltoum *et al.* 2001). The reaction is partly reversible (Eltoum *et al.* 2001; Grizzle *et al.* 2008), which means that autolytic enzymes in the tissue and bacterial enzymes in the digestive tract may become active again if the ethanol concentration drops below 50–60% for a longer time. Observations on insects suggest deterioration within 10 years at ethanol concentrations below 90% (Salmon 1947). Ethanol as single preservative may also cause "considerable distortion of the micro-anatomy in pieces of animal tissue"; therefore, ethanol has been applied as fixative together with, e.g., formaldehyde and acetic acid (often abbreviated, but not always explained, as FAA = formalin + acetic acid + alcohol or AFA = alcohol + formalin + acetic acid) with acetic acid opposing the shrinkage (Pritchard & Kruse 1982, p. 124; Nowacek & Kiernan 2010, p. 145). In marine species, the salts from the seawater may precipitate on the specimens as a crust and inhibit proper fixation if the latter are preserved in ethanol of high concentration (Gatenby & Beams 1950, p. 21; Steedman 1976b, p. 176). This may be overcome by acidifying the ethanol with hydrochloric acid or another acid (Gatenby & Beams 1950, p. 21). It has to be kept in mind that many "fixatives", e.g., a mixture of methanol and acetic acid (Saito *et al.* 1993), are not intended to conserve the structure of the inner organs of a specimen, but to preserve just the chitinous exoskeleton for microscope observation. Such cocktails as well as reversible coagulating agents such as ethanol should not be regarded as fixatives *sensu stricto* like the cross-linking agents formaldehyde and glutardialdehyde.

Various fixatives and preservatives. Bracegirdle (1978, p. 59) dated the "beginning of adequate fixation" for microscopy to the application of chromium trioxide as a hardening agent by Jacobsen in 1833 (Tab. 1). Mercuric chloride (= corrosive sublimate of older literature) was used first by Blanchard since about 1846 and survived in many recipes like Heidenhain's Susa, Zenker's fixative, and Gilson's fluid (Tab. 1; Gray 1954; Bracegirdle 1978, pp. 60–62; Böck 1989; Kiernan 2015). Material treated with fixatives containing mercuric chloride is prone to the formation of crystals, which may be removed by washing with alcoholic iodine and sodium thiosulfate solution (Gray 1954; Kiernan 2015, pp. 18, 70). Acetic acid was applied first in microscopy by Clarke in 1851, osmium tetroxide by Schultze in 1864, and picric acid (= trinitrophenol) by Ranvier in 1875 (Tab. 1; Bracegirdle 1978, pp. 61–62). This range of fixatives was occasionally used alone or in combination with other fixatives like formaldehyde for museum material (Neuhaus pers. obs. from catalogue data at Museum für Naturkunde Berlin), but their main application seems to have been related to histological studies.

It may be tempting to preserve specimens intended for both molecular and morphological studies in DESS, consisting of 20% dimethyl sulfoxide (= DMSO), 0.25 M disodium ethylene diamine tetraacetic acid (= disodium EDTA), and saturated sodium chloride (Yoder *et al.* 2006). Whereas the DMSO facilitates penetration of smaller molecules, disodium EDTA and sodium chloride inhibit nucleases, which can degrade DNA (Naem *et al.* 2010). In this specific case, marine, terrestrial, and parasitic nematodes were initially preserved in DESS, mounted in glycerol, and studied with a light microscope after five months (Yoder *et al.* 2006). However, DESS does not initiate cross-linking of the tissue, so once a specimen is mounted, its morphology is not maintained by a preservative anymore. It remains open, how long such a specimen may survive as a slide mount beyond the five months tested. Also, crystals may form in some specimens (Naem *et al.* 2010).

TABLE 1. Timeline of the introduction of selected new techniques for microscope slides.

Time	Inventor	New technique	Source
mid 17th century		observation of specimens in air, water, or olive oil	Bracegirdle 1978, pp. 8–10
ca. 1662	Boyle	ethanol introduced as preservative	Reid 1994, p. 32
late 17th century		specimens mounted between 2 pieces of mica on vertically oriented slider made of ivory or hardwood	Bracegirdle 1978, p. 18, text-fig. 4; Gill 2013, p. 42
1789	Ingen-Housz	homemade glass coverslip introduced in order to prevent evaporation of liquid observation medium	Bracegirdle 1978, pp. 23, 113–114
1795	Ypelaar	first resin mount with Venetian turpentine	Bracegirdle 1978, p. 88
1833	Jacobsen	chromium trioxide introduced as fixative	Bracegirdle 1978, p. 59
1830 or 1835	Bowerbank or Pritchard	Canada balsam introduced as mounting medium	Bracegirdle 1978, p. 88
ca. 1835		homemade glass coverslip in common use	Bracegirdle 1978, pp. 113–114
1839	provisional committeee of the later Royal Microscopical Society of London	fixation of size of microscope slide to 3 x 1 inches and to 3 x 1.5 inches	Bracegirdle 1978, pp. 111–112; Gill 2013, p. 42
1840	Chance Brothers	glass coverslips commercially produced	Bracegirdle 1978, pp. 113–114
about 1843		glass coverslip used in combination with turpentine-cleared specimen mounted in balsam	Bracegirdle 1978, pp. 23, 113–114
1846	Blanchard	mercuric chloride introduced as fixative	Bracegirdle 1978, pp. 60–62
1849	Warington	glycerol introduced as mounting medium	Bracegirdle 1978, p. 93
1851	Clarke	acetic acid introduced as fixative	Bracegirdle 1978, pp. 61–61
1852	Deane	glycerol-gelatin introduced as mounting medium	Bracegirdle 1978, p. 93
1864	Schultze	osmium tetroxide introduced as fixative	Bracegirdle 1978, pp. 61–61
1869	Klebs	paraffin introduced as embedding medium in histology	Bracegirdle 1978, p. 77
1875	Ranvier	picric acid introduced as fixative	Bracegirdle 1978, pp. 61–61
1875	Lavdowsky	chloral hydrate introduced	Bracegirdle 1978, p. 93
1882	Hoyer	gum-chloral medium introduced as mounting medium	Hoyer 1882; Upton 1993
1883	Van Heurck	Styrax introduced as mounting medium	Bracegirdle 1978, p. 92

... ...continued on the next page

TABLE 1. (Continued)

Time	Inventor	New technique	Source
1893	Blum	formaldehyde introduced as fixative	Blum 1893
1906	Gilson	Euparal introduced as mounting medium	Gilson 1906
1917	Cobb	Cobb aluminum double-coverslip slide introduced	Cobb 1917
1927	Hanna	Hyrax introduced as mounting medium	Hanna 1927
early 1930s		Caedax introduced as mounting medium	e.g., Diederichs 1932–1933
1933	Perruche	Coumarone introduced as mounting medium	Perruche 1933; Frison 1952a
1935	Thorne	Thorne ringing compound (= Glyceel, Zut) introduced as coverslip seal	Thorne 1935
1938	Scheuermann & Tauböck	Celochloral introduced as mounting medium	Ant 1957; Hirling 1957–1958
1939	Kirkpatrick & Lendrum	DPX introduced as mounting medium	Kirkpatrick & Lendrum 1939
1942	Lubkin & Carsten	poly(vinyl alcohol) introduced as mounting medium	Lubkin & Carsten 1942
1943	Fleming	Naphrax™ introduced as mounting medium	Fleming 194
ca. 1944	Shillaber	Aroclors® introduced as mounting media	Frison 1955
1949	Hanna	Pleurax introduced as mounting medium	Hanna 1949
1950	Clark & Morishita	C-M Medium introduced as mounting medium	Clark & Morishita 1950
1968	Travis	Glyptal introduced as coverslip seal	Travis 1968
1996	Sabir	Corseal introduced as coverslip seal	Sabir 1996
2013	Villani *et al.*	Visikol™ introduced as mounting medium	Villani *et al.* 2013

In search for fixatives less toxic for humans, a range of different chemicals has been tested. Among these is acidified Lugol solution (iodine, potassium iodide) for preserving plankton organisms (Jaspers & Carstensen 2009). However, copepods shrink by 17% within the first 36 hours, Lugol solution stains specimens intensely, the iodine-based solution deteriorates on exposure to light, and may be absorbed by plastic material. It remains to be tested how long specimens may last if initially preserved with Lugol solution and whether taxonomically important characters can be recognized in sufficient detail or not.

Formaldehyde. Formalin, a solution of the gas formaldehyde in water, was introduced as a fixative by Blum in 1893 (Tab. 1; Blum 1893; Bracegirdle 1978, p. 62; Böck 1989, p. 84; Reid 1994, p. 54). Therefore, especially older parts of a museum collection were most probably preserved in ethanol only (but see also previous paragraph), which may lead to conservation problems if slides are mounted from such material. Unfortunately, the relevance of proper fixation with formaldehyde (see below) has been also ignored by later workers until today, where there is often the focus on preserving material for molecular studies and depositing voucher specimens in a museum collection in the same preservative, viz, usually ethanol.

Formaldehyde dissolves in aqueous solutions to methylene hydrate, which reacts with several functional groups of proteins to unstable hemiacetals and related adducts (Kiernan 2015, pp. 24–27). The free hydroxymethyl groups of the hemiacetal-like adducts react with other functional groups of proteins and cross-link the latter via stable methylene bridges thereby constituting the fixing capability of formaldehyde (Kiernan 2015; Nowacek & Kiernan 2010, pp. 142–143). A plateau of binding of formaldehyde to proteins is reached after 24 hours. The cross-linking process seems to take 1–6 weeks depending on the size of the specimen, so fixation for about one to two weeks is recommended for morphological studies of small- to medium-sized specimens (see also Jones 1976, pp. 155–168; Sanderson 1994, p. 20; Kiernan 2015, pp. 24–28, 2000; Nowacek & Kiernan 2010, pp. 142–143). Eltoum *et al.* (2001) and Grizzle *et al.* (2008, p. 58) doubted that the cross-linking initiated by formaldehyde during fixation for up to 24 hours contributed most to the fixing capability of formaldehyde and suggested denaturing of macromolecules by the formation of hydroxymethyl groups as the major effect. In any case, fixation with formaldehyde over about two weeks is less reversible (Sanderson 1994; Kiernan 2015; Nowacek & Kiernan 2010) and is, therefore, preferred over fixation for a single day or fixation with ethanol. For histological sectioning, formaldehyde seems still to be unsurpassed by other fixatives despite the extensive efforts to substitute it and despite its cancerogenic potential (Buesa 2008).

Hooper (1986b) recommended storing nematodes for not longer than one year in TAF, an aqueous mixture of formaldehyde and triethanolamine, in order to avoid artifacts in the cuticle. Huber (1998, p. 377) claimed that formaldehyde would "erode" the cuticle of nematodes and arthropods, if specimens were stored in this chemical. However, from the following text it becomes clear that Huber (1998, p. 380) referred to fixing and not to storage for an extended amount of time. This statement is a bit amazing, because formaldehyde in combination with other chemicals is still recommended for nematodes (e.g., Hooper 1970, 1986a, 1986b; Hooper *et al.* 2005).

3.1.2 Chemical maceration

Internal organs of small invertebrates are often macerated, viz, chemically destroyed, and only the cuticular exoskeleton studied by light microscopy. This classical process has been confused with "clearing" in numerous publications. However, clearing is a totally different, physical phenomenon that, unlike chemical maceration, does not destroy the structural integrity of a specimen (see chapter 3.1.3 Physical clearing). To complicate matters even further, "clearing" in histology also means clearing from water; this refers to using non-polar liquids like benzene, chloroform, and xylene as intermediate agents between dehydrating agents such as ethanol and paraffin wax or resinous mounting media (Pritchard & Kruse 1982, pp. 121–122; Kiernan 2015, pp. 2, 53).

Maceration may also affect the cuticle and soften it. Maceration seems to be necessary for Thysanoptera, because "unmacerated specimens of pale species tend to produce an opaque film over their body surfaces which obscures detail within about ten years" (Mound & Pitkin 1972, p. 122). Sometimes, degreasing of a specimen in a mixture of ethanol and either xylene and ethylacetate or diethyleether is necessary in order to avoid interaction of the specimen and mounting medium, e.g., in aphids (Heikinheimo 1988). Brown (1997) and Brown & Boise (2005, 2006) noted for insects that the "specimens preserved in formalin cannot have their body contents removed and so are almost useless for taxonomic studies". A similar statement was given by Mitchell & Cook (1952) and by

Jeppson *et al.* (1975, p. 385) for freshwater and phytophagous mites correspondingly, by Bartsch (1988) for marine Acari, and by Gardner (1975) for plants. These statements for some invertebrates and plants are certainly not true for all invertebrates, because Kinorhyncha have been regularly preserved in formaldehyde and subsequently mounted in Hoyer's mounting medium with total removal of the inner organs (e.g., Higgins 1969, 1983, 1988, 1990; Neuhaus pers. obs.). For insects stored in ethanol of high concentration for a longer period of time, maceration will be facilitated by keeping the specimen in 50% or a lower concentration of ethanol for a few days before maceration (Mound & Pitkin 1972, p. 125). Marine and freshwater mite exoskeletons seem to require, or at least benefit from, removal of internal organs by digestion with the enzymes pepsin or trypsin (Newell 1947; Mitchell & Cook 1952; Bartsch 1988).

Maceration, bleaching, and de-waxing processes in arthropods often include rigorous procedures with chloral hydrate, eau de Javel (= sodium hypochlorite, NaClO), glacial acetic acid, hydrochloric acid, lactic acid, potassium or sodium hydroxide, tetrahydrofuran, and xylene (the latter two both for de-waxing), sometimes done at 80°C or even 100°C in order to accelerate the processes (Fig. 13; Behrens 1892, pp. 68–70; Nye 1947; Salmon 1947; Essig 1948; Lambers 1950; Wagstaffe & Fidler 1955, pp. 177–178; Richards 1964; Lersten 1967; Singer 1967; Wirth & Marston 1968; Eastop & Emden 1972, p. 4; Mound & Pitkin 1972; Barbosa 1974; Gardner 1975; Rusek 1975; Martin 1978, 1999; Bink 1979; Noyes 1982; Pritchard & Kruse 1982; Thatcher 1987; Wilkey 1990; Saito *et al.* 1993; Upton 1993; Amrine & Manson 1996; Famadas *et al.* 1996; Brown & Boise 2005, 2006; Faraji & Bakker 2008). Supposedly, "the use of cold caustic over a more or less prolonged period gives more uniform results than relatively short immersion in hot or boiling caustic" (Hardwick 1950, p. 231). Glacial acetic acid also dehydrates specimens and has been commonly used as a combined macerating and dehydrating agent for mounting in a non-aqueous medium (Spence 1940d; Richards 1964; Brandham 1970; Berland 1984). At the same time, acetic acid renders specimens supple with their appendages expanded.

Chloral hydrate (Fig. 13) has been, and still is, applied to specimens alone or as part of a gum-chloral medium or phenol. Chloral hydrate decomposes in aqueous solution within 15 weeks lowering the pH from 6.72 to 4.75–2.33; this process is accelerated by UV irradiation and the pH may drop from 6.25 to 1.6 in 10 hours (Luknitskii 1975, p. 260). Chloral hydrate substance has sedative properties, is regulated at least in the USA, and may be difficult to obtain, but "many pharmacopeias [...] have published protocols for microscopic authentication analyses of herbal preparations using acidified chloral hydrate [...] as clearing agent" (Villani *et al.* 2013). Therefore, the latter authors suggested replacement of chloral hydrate by Visikol™ (for more details see chapter 3.7.21 Visikol™).

Sometimes, several macerating and clearing agents are applied at the same time resulting in a very powerful process like in Herr's four-and-a-half clearing fluid for plant material consisting of chloral hydrate, clove oil, lactic acid, phenol, and xylene (Herr 1971). For maceration and/or de-waxing, a mixture of chloral hydrate and phenol is recommended (Spence 1940a; Lambers 1950; Ossiannilsson 1958; Eastop & Emden 1972; Rusek 1975; Martin 1999). This mixture has also been suggested as a mounting medium, but preparations do not last very long because of the rapid evaporation of both substances (see also Tab. 10; Spence 1940a). A mixture of lactic acid and phenol called lactophenol is applied frequently (Denham 1923; Richards 1964; Singer 1967; Eastop & Emden 1972; Esser 1974; Bink 1979; Huys & Boxshall 1991; Upton 1993; Brown & Boise 2005; Faraji & Bakker 2008). Essig's Aphid Fluid contains glacial acetic acid, lactic acid, phenol, water, and originally also ethanol (Essig 1948; Wilkey 1990). Martin (1999, p. 124) used a mixture of phenol with Histoclear or xylene for de-waxing and bleached specimens in ammonia and hydrogen peroxide (Martin 1999, pp. 124–125). Martin (1978, pp. 108, 166) suggested Nesbitt's fluid, a mixture of chloral hydrate, water, and hydrochloric acid, for macerating mites and insects. Additional formulae may be found in Gray (1954, pp. 177–178).

Great care must be taken that the specimen is washed repeatedly and none of these and related substances as well as the primary fixative remain in the specimen, because the chemicals will influence long-time stability of the mount (see chapter 3.7.25 Discoloration; Essig 1948; M. Ipe and B. Pitkin in Disney & Henshaw 1988). However, recipes provided by numerous authors and the frequent statement that specimens may be mounted directly from the macerating agent indicate that the necessity of carefully washing off the macerating agent is all too often ignored entirely. In these cases, the macerating agent remains in the specimen and becomes part of the mounting medium—with all its disastrous consequences. Brown (1997, p. 11) stated that "the NHM has stopped the practice of clearing specimens in chloral phenol before mounting in gum chloral mountants and will continue and increase the use of Euparal and Canada balsam", so there is some hope for change at least in some institutions.

Whereas classical maceration results in complete destruction of soft tissues, more recent efforts in vertebrate studies concentrate on removal of lipids and decolorization of tissues, especially hemoglobin, myoglobin, and other pigments, by treatment with two cocktails (reagent 1: N,N,N',N'-tetrakis(2-hydroxypropyl) ethylenediamine, polyethylene glycol mono-*p*-isooctylphenyl ether, Triton X-100, urea; reagent 2: sucrose, 2,2',2''-nitrilotriethanol, Triton X-100, urea) for study of entire specimens with light-sheet microscopy (e.g., Susaki *et al.* 2014; Tainaka *et al.* 2014). A similar approach using the sugar alcohol xylitol (= pentanpentol), the detergent sodium deoxycholate, and urea was followed for whole-plant studies by extracting chlorophyll and therefore diminishing its autofluorescence (Kurihara *et al.* 2015). All these efforts also aim at minimizing the mismatch in refractive index between coverslip, observation medium, and specimen.

3.1.3 Physical clearing

Stained or unstained tissue is "cleared" by a clearing agent that remains in the specimen during mounting and is part of the permanent mount or represents the mounting medium in the case of glycerol. The classical clearing is a physical phenomenon and acts via the refractive index of the clearing agent being close to that of the specimen; therefore, structural characters of a specimen become less visible, whereas stains stand out optically, which is particularly useful for stained histological sections (comp. chapter 3.7.3 Refractive index of mounting media; Lillie *et al.* 1953, p. 57; Krauter 1983; Kiernan 2015, p. 53, tab. 4.1; Ravikumar *et al.* 2014). If a specimen is intended for mounting in a hydrocarbon-soluble medium, aniline oil (toxic, carcinogen), anise oil, benzene, benzyl benzoate, bergamot oil, cajeput oil (= cajuput oil), cedarwood oil, cinnamon oil (= cassia oil), clove oil, creosote (a mixture of various phenols), isoamyl acetate, limonene, methyl benzoate (= Niobe oil), methyl salicylate (= wintergreen oil), origanum oil, phenol, terpineol (= artificial oil of lilac), *tert*-butyl alcohol, or oil of turpentine (applied first about 1843) may be used for clearing the specimen (Fig. 13; Behrens 1892, pp. 68–70; Spence 1940d; Fox 1942; Doetschman 1944; Gatenby & Beams 1950, pp. 66–70; Hardwick 1950; Wagstaffe & Fidler 1955, pp. 173–174; Wirth & Marston 1968; Heming 1969; Barbosa 1974; Bracegirdle 1978, p. 82; Martin 1978, p. 129; Palma 1978; Pritchard & Kruse 1982; Krauter 1983; Berland 1984; Böck 1989; Sanderson 1994, p. 43; Brown 1997; Kiernan 2015; Gill 2013, pp. 248–249).

Oil of turpentine should not be confused with Venetian turpentine (= Venice turpentine) as in GESTIS Substance Database (Available from: http://gestis-en.itrust.de/nxt/gateway.dll/gestis_en/000000.xml?f=templates$fn=default.htm$3.0, accessed 14 January 2016). Oil of turpentine contains the volatile substances distilled from the resin of various species of *Pinus* and consists of various liquid monoterpenes like α-pinene, ß-pinene, Δ^3-carene, limonene, myrcene, and ß-phellandrene; Venetian turpentine originates from the resin of *Larix decidua* Linné, 1758 and contains different resinous pimaradiene, abietadiene, and abietatriene acids (Tab. 5; Mills & White 1999, pp. 95, 100–102). The unsaturated hydrocarbons of oil of turpentine "tend to react with oxygen in the air, becoming increasingly polar with the formation of peroxides [...]. These can then polymerize to form insoluble and yellow products. These oxidized components will in turn cause any solutes, such as other polymers, to oxidize, deteriorate and cross-link. [...] For that reason, oil of turpentine is not recommended to be used for conservation purposes" (Horie 2011, pp. 241242). Although this statement is given for picture varnishes, there may be some truth also for microscope slides.

Phenol also dehydrates specimens and has been commonly used as a combined clearing and dehydrating agent since at least 1873. In addition, phenol renders specimens supple with their appendages expanded (Spence 1940d; Fox 1942; Wirth & Marston 1968; Berland 1984; Famadas *et al.* 1996; Brown 1997, p. 8). Opposite to xylene, cedarwood oil is also supposed not to harden specimens (Pritchard & Kruse 1982, p. 122). Clove oil makes specimens brittle and causes darkening of Canada balsam over time (Behrens 1892, p. 69; Gatenby & Beams 1950, p. 67; Gray 1954, p. 56; Wagstaffe & Fidler 1955, p. 173). Histoclear and other xylene substitutes using limonene are reported to harden tissues like brain, liver, and spleen considerably more than substitutes based on alkanes, although the alkane-based Clear-Rite™ 3 is known to harden tissues, too (Buesa & Peshkov 2009, Tabs 2–3, p. 250). Alkanes are "usually incompatible with xylene or toluene-based mounting media" (Buesa & Peshkov 2009, p. 250).

Mounting a specimen in glycerol also clears tissues (see chapter 3.7.4 Liquid mounting media (formaldehyde, lactophenol, glycerol, Zeiss W15); Morse 1992, p. 3).

3.1.4 Staining

Meiofauna is easier to recognize during sorting, and this process consequently accelerated if specimens are stained with Rose Bengal binding to proteins for 10 minutes up to two days (Pfannkuche & Thiel 1988, p. 143). Arthropods are often stained with acid fuchsin, basic fuchsin in ammonia, borax carmine, cibacron turquoise blue GE, chlorazol black E, eosin, Evans blue, fast green, light green, lignin pink, methyl blue, orange G, phenol-fuchsin (= Ziehl's carbol-fuchsin), procion blue 3GS, safranin, and toluidine blue; specimens are often, but not always, macerated before staining (Imms 1929, pp. 167–168; Hardwick 1950; Heinze 1952; Mitchell & Cook 1952; Evans & Browning 1955; Knudsen 1966, p. 274; English & Heron 1976; Hockin 1981; Huys & Boxshall 1991, p. 450; Saito *et al.* 1993; Koomen & Vaupel Klein 1995; Amrine & Manson 1996; Martin 1999, p. 125; Faraji & Bakker 2008). The stain has to be compatible with the clearing medium and the mounting medium if the former is used at all (Tab. 6; Brown 1997, p. 5). Acanthocephala and other invertebrates may be softened and made more permeable for borax carmine or hematoxylin staining by soaking in trisodium phosphate for some time (Van Cleave & Ross 1947b).

English & Heron (1976, p. 288) advocated fixation of copepods in formaldehyde and the emulsified version of the antioxidant dibutylhydroxytoluene (= butylated hydroxytoluene, BHT, trade name Ionol CP-40) in order to preserve specimens "in a supple and cleared condition". This idea was originally applied for improved color retention in fish (Waller & Eschmeyer 1965). English & Heron (1976) further suggested macerating specimens in lactic acid and stain copepods with solophenol blue 2RL (= chlorantine fast blue 2RLL), which binds to chitinous structures slowly and selectively, so the reaction can be stopped when appropriate. This dye behaves different from many other dyes, which stain meiofauna so rapidly and intensively even at low concentrations that specimens are usually overstained (English & Heron 1976).

Unfortunately, stains may also mask some morphological characters and make light microscope investigations with differential interference contrast more difficult (Huys & Boxshall 1991, p. 450; Koomen & Vaupel Klein 1995; Sørensen & Pardos 2008; Neuhaus *et al.* 2013), so staining cannot be recommended at all for morphological studies of meiofauna. Possibly, investigating specimens with a microscope equipped with phase contrast or differential interference contrast (Martin 1978, p. 136) or emulating various contrasting methods with software (see chapter 3.12.1 Microscope equipment; Barone-Nugent *et al.* 2002; Cody *et al.* 2005) may substitute or at least complement staining.

3.2 Storage

Brown (1997, p. 6) stated correctly that "microscope slides require a controlled environment" and suggests "four lines of defense—the sealant ring, the envelope, the cabinet and the room in which the cabinet is housed". These topics are discussed below. Slides can be stored vertically or horizontally, in cabinets, in boxes, on trays, enwrapped with (often paper-based) materials of varying quality, etc. (Brown 1997; Gütebier 2011). Reasons for a certain way of storage will often be related to historical measurements. It is suggested here to evaluate the current situation of storage, set priorities depending on finances and personnel, and to improve the situation step by step until a satisfactory stage is reached (Tab. 14).

It must be clearly stated that microscope slides should always be stored separately from other biological specimens and not, e.g., together in boxes with pinned insects, because storage requirements of different materials may be different (see also chapter 3.4.2 Variety of slides; Brown 1997). Canada balsam is reported to blacken and to turn opaque, if mounts are stored in insect boxes treated with parachlorobenzene (= 1,4-dichlorobenzene) for pest control (Halliday 1994).

3.2.1 Environmental factors

Temperature, relative humidity, light, oxygen, dust, and volatile compounds represent the main environmental factors affecting microscope slides. Based on a review about accelerated ageing of paper, film, and magnetic media, Michalski (2002, p. 70) gave the following general statement about ageing of museum artifacts: "Things in museums that suffer inherent from chemical decay can be assumed to last approximately twice as long for each 5°C

drop in storage temperature. [...] Organic materials suspected of suffering acid hydrolysis, such as paper, film and textiles acidified by pollution, can be expected to last more than twice as long at 35% RH as compared to 70% RH [...]". This certainly also has some truth for slides, but other aspects have to be considered, too.

Grimaldi (1993, p. 45) observed that Baltic amber exposed for about four decades to a temperature of 32–35°C during summer darkened considerably. Oxygen was supposed to contribute to the deterioration of amber as well (Thickett *et al.* 1995) and has been demonstrated to actually do so by chemical analyses (Pastorelli 2011; Pastorelli *et al.* 2012). Exposure of amber of various origin to accelerated aging by light, heat, and fluctuating humidity over one year lead to increased visible or only spectrophotometrically detectable deterioration of the amber, including crazing of the surface, cracking, and darkening; Bisulca *et al.* (2012) favored especially a stable relative humidity as prerequisite of long-term storage of amber. An accelerated aging study at 70°C contributes to understanding the mechanisms, by which the polymer degrades (Pastorelli *et al.* 2013). Mounting in a mixture of gum dammar and Venetian turpentine (Klebs 1880, pp. 24–25) seems to stabilize Baltic amber at the Museum für Naturkunde Berlin now for more than 130 years now (Neumann 2010).

The Museums and Galleries Commission (1992) suggested that slide collections should be stored in rooms with little variation of temperature at about 18°C but did not provide any further argumentation for this recommendation. Changes in temperature may lead to cracking of slide mounts because of the different coefficients of thermal expansion of glass, mounting medium, and coverslip seal. Therefore, it is not recommended storing slides at a lower temperature than 18°C, because otherwise extra caution has to be taken for equilibration to the higher temperature and higher humidity in the area where slides are studied. A repeated change in humidity would also accelerate glass deterioration (Davison 2003, p. 192).

Allington & Sherlock (2007a, 2007b) reported for the Zoology Department of the Natural History Museum at London that the temperature of the microscope slide rooms ranged from 18–27°C and the humidity from 37–67% during one year. At the Museum für Naturkunde Berlin, the old hall where microscope slides of the worm collection were stored from about 1918 to 2010 possesses large windows to the north and to the south. Box-windows with two panes unrestored for many decades and aluminum sunblinds (since 1997) somewhat reduce temperature exchange between outside and the building, but are not very efficient. Consequently, temperature varied from 14–28°C and relative humidity from 33–51% during the year based on data logging from January 2012 to April 2013 (data courtesy of C. Lüter). Nowadays, these slides are stored in a neighboring hall with windows to the north, which were restored fulfilling German standards for energy saving (= Energieeinsparverordnung 2009 = EnEV 2009). Annual fluctuation of temperature and relative humidity are reduced now (Neuhaus pers. obs.). The relative humidity is suggested to be kept at 60% (Museum and Galleries Commission 1992), but Sterflinger (2010, p. 49) stated that "in museums the range of 55% RH is generally regarded as the border line for fungal growth and thus climate control is adjusted below this value". She also emphasized that microclimates within collection halls favor fungal growth in poorly ventilated areas and on "walls with temperatures below the dew point". Weintraub *et al.* (1995) claimed that a humidity above 70% may cause mold and bacteria to grow and water-based mounting media to take up moisture if the coverslip seal is not functional anymore. On the contrary, a very low humidity may lead to evaporation of water from water-based mounting media and discoloration (Brown 1997).

Slides should be protected from light, which may cause the inscriptions on the label and the stains of histological sections to fade (Barr 1970; Dewse & Potter 1975; Goodway 1995; Brown 1997) and the mounting media to deteriorate (Denham 1923; Brown 1997; Jersabek *et al.* 2010). Exposure of amber to daylight behind window glass does show a severe deleterious effect on amber (Pastorelli *et al.* 2011). Dust should be kept to a minimum (Goodway 1995), because removing the dust from a slide requires additional handling of the preparation with the risk of damaging the mount and the label. Cabinets should be dust-proofed as much as possible. Plastic seal tubing as used for sealing windows and brush seals used for doors may represent an option to close wider slits at the doors of cabinets. However, these "seals" may favor the formation of unwanted microclimate conditions especially concerning volatile compounds in a cabinet (see below).

3.2.2 Cabinets

Cabinets for microscope slides are usually made of wood or of metal. While wooden cabinets offer the advantage of easy availability, the material itself as well as the adhesives, paints, and varnishes used in the construction of the cabinet will inevitably produce volatile acids and aldehydes leading to corrosion and other damage of the items the

cabinet is meant to protect (Hatchfield 1995; Tétreault & Stamatopoulou 1997). Acids originate from the on-going hydrolysis of the hemi-celluloses of the wood and are generally produced at a higher degree in hardwoods such as oak than in softwoods such as pine. Formaldehyde derives from the wood itself and from the resin used to make plywood and particleboard (Hatchfield 1995; Gibson & Watt 2010). Volatile acetic acid may also originate from paints and varnishes used on cabinets (Tétreault & Stamatopoulou 1997). Emission of acids increases with increasing relative humidity and temperature (Gibson & Watt 2010). Exposure of amber to acetic acid, formic acid, and hydrogen sulphide for a couple of hours to three weeks resulted in crazing and exfoliation (Waddington & Fenn 1988; Williams *et al.* 1990) indicating deleterious effects also for mounting media based on natural resins. In the National Museums of Scotland, glass artifacts developed crystalline deposits on their surfaces because of evaporation of acetic acids (1,193–2,019 µg x m^{-3}), formic acid (366–520 µg x m^{-3}), and formaldehyde (34–858 µg x m^{-3}) from the cabinets in conjunction with fluctuating temperature and relative humidity (Robinet *et al.* 2004).

Acids and aldehyde are especially dangerous for the slides, because a cabinet constitutes a closed environment. This effect may be minimized by good ventilation of the collection room, by gas sorbents placed in the cabinet such as activated charcoal (= activated carbon) with or without a molecular sieve (= molecular trap, scavenger), and by vapor barriers (Grzywacz & Tennent 1994; Hatchfield 1995; Weintraub *et al.* 1995; Shashoua 2008, pp. 196–201). Better ventilation may cause more input of dust, so precautions have to be taken to reduce the dust load. Acidic, lignin-containing or historic papers benefit from molecular sieves and active carbon in terms of degradation (Strlič *et al.* 2011). Barriers may be made of aluminium plates or foils. We would not expect a considerable contribution of such barriers to lowering the concentration of organic volatiles from the wood in a cabinet with numerous drawers as usually exist in slide collections, because there is both not enough space for such barriers, and the surface of the wood is too high in a slide cabinet to be entirely covered with a barrier. Volatile acids in a cabinet may be monitored roughly with an easy and cheap method during a short period of time (24 hours) with acid-detecting strips or for a longer time such as a month simultaneously with a silver, copper, and lead coupon (Shashoua 2008, pp. 199–201; Coughlin 2011). The silver coupon reveals corrosion by volatile hydrogen sulfide or carbonyl sulfide, the copper coupon reacts with chlorides, oxides (SO_2, NO_X), and sulfur compounds, and the lead coupon shows corrosion mainly by aldehydes and acetic gases (Grzywacz 2006). This so-called Oddy test was developed for testing materials for the storage and display of museum objects in the early1970s by Antony Werner and Andrew Oddy. The test takes place originally in a sealed container including one or all three coupons, distilled water, and a sample of the material to be tested for volatile substances for 28 days at 60°C (see Robinet & Thickett 2003). Wang *et al.* (2011) used metal films instead of solid metal coupons and reduced testing time to 14 days.

A metal cabinet with a stoved or baked enamel coating based on powdered paint electrostatically applied to the metal surface seems to represent the most serious and recommendable alternative to wooden cabinets (Tab. 14; Hatchfield 1995). If produced properly, volatile substances will originate at significantly lower concentrations from a metal cabinet (Grzywacz & Tennent 1994). The National Museum of Natural History, Washington D.C., Smithsonian Institution used to store its slides horizontally on metal trays in a steel cabinet "Lab-aid" made by The Technicon Company, New York (Fig. 1F–H). Unfortunately, this cabinet is not produced anymore. Nowadays, Delta Designs Ltd, Topeka, Kansas, produces metal slides cabinets, which may even be custom-designed (Available from: http://www.deltadesignsltd.com/, accessed 21 September 2016). This cabinet also has a mechanically attached silicon gasket all around the door opening. Metal "Fisherbrand™ micro slide storage cabinets" by Fisher Scientific allow only vertical storage of slides (Fig. 1I) and are, therefore, not recommended for museum collections. Alternatively, a metal cabinet with drawers not higher than 50 mm meeting archival-quality standards for museums may be adapted for slides, but trays would have to be custom-made.

3.2.3 Trays

Horizontally stored microscope slides may be located on trays made of cardboard, wood, plastic, or aluminum (Goodway 1995). Among these, anodized aluminum (Hatchfield 1995) seems to the authors of this paper to represent the longest lasting material of least potential impact on the slides and its environment (Tab. 14). One of the best designed systems the senior author has come across are the trays of Technicon (Fig. 1F–H) and of Delta Designs in a metal cabinet (see also chapter 3.2.2. Cabinets). Each steel tray of Technicon with a grey hammer finish enamel for a neutral background holds a series of 16 or 32 standard slides in a row, whereas the tray of Delta

Designs comes with a white finish and holds up to 66 standard slides. Each slide can be picked easily with the help of a hole at the bottom of the tray (Fig. 1G for Technicon). Oversized slides may be stored by bending down the half-rounded holders (Fig. 1G, arrowheads). A similar system but in a wooden cabinet is documented by Martin (1978, fig. 108).

Archive-quality cardboard seems to be the most reasonable alternative to aluminum (see also Gütebier 2011). These would have to be custom-made. One company devoted to high standards in paper articles in Germany and able to provide custom-made items of archive-quality is Klug Conservation (Tab. 12). In the long run, wood is not regarded as a good choice for trays (see also discussion about cabinets). Also, many plastics are not of archival quality, because they contain plasticizers (up to 20–50%), UV inhibitors, dyes, and other additives, which may gas-off or deteriorate over time, or the plastics are coated on the surface (Sturm 2006; Shashoua 2008, p. 58). Several factors accelerate natural deterioration of plastics in the presence of oxygen, viz, UV-light, increased relative humidity above 50%, heat, stress, and pollutants like ozone, sulfur dioxide, and formaldehyde (Blank 1990; Oosten 2002b). Deteriorating plastics can be recognized visually by "cracking, warping, crazing, delamination, liquid surface deposits, solid surface deposit, discoloration and crumbling", olfactory by "a distinctive smell", and tactilly by "a sticky surface" (citations from Oosten 2002b; Shashoua 2008). Sturm (2006) stated that "there are five plastics that are considered archival", viz, polyethylene, polypropylene, polycarbonate, poly(ethylene terephthalate), and polytetrafluoroethylene. A series of (partly destructive) tests allows distinguishing certain clear plastics from each other, but this will probably be useful only if larger amounts of the same plastics are used (Williams *et al.* 1998; Oosten 2002b).

3.2.4 Horizontal *versus* vertical storage

Microscope slides should always be stored horizontally rather than vertically for the following reasons, although the former takes up more space (Tab. 14; Wagstaffe & Fidler 1955, p. 197; Eastop & Emden 1972; Martin 1978; Pritchard & Kruse 1982; Gutierrez 1985; Hooper 1986a, 1986b; Brown 1997, 1998; Allington & Sherlock 2007a, 2007b):

(1) Mounting media such as Canada balsam or Euparal require many months to dry in the center of the slide and about six weeks if kept in an oven at 40°C (Mound & Pitkin 1972). It seems that at least in some cases, the mounting medium, probably Canada balsam, remains to some extent viscous even after years of storage (Spence 1939; Brunner & Blueford 1986; Gudo *et al.* 2012). If stored vertically, both the specimen and the mounting medium may move under the coverslip (Martin 1978: p. 116; Pritchard & Kruse 1982). It cannot be excluded that supposedly "sufficiently hardened" mounting media may creep slowly under the influence of gravity over decades if stored vertically (Clarke 1941; Goodway 1995; Brown 1997; Gütebier 2011). Brown (1997, 1998) mentioned that slides were baked in an oven at 30–40°C in order to harden the mounting medium prior to vertical storage but did not indicate any time schedule. Slides with a liquid mountant such as glycerol-paraffin slides should under all circumstances be stored horizontally (Brown 1997, 1998), because the slides will rapidly lose the liquid content should the paraffin detach locally from the coverslip and if the seal leaks.

(2) The coverslip may detach from the mounting medium and remains on the slide only if the slide is stored in a horizontal position.

(3) The adhesive of a label may dry out, such that the label detaches and collects somewhere if slides are stored vertically (Goodway 1995). In this way, a slide may lose the primary information, which makes the preparation valuable scientifically.

(4) Inspection of vertically stored slides takes considerably more time and effort, because each slide has to be taken into the hand and checked individually. In horizontally stored slide collections, problems with a slide can be seen at a glance without even touching the preparation (comp. Fig. 1C–E, G with Fig. 1I).

Vertically stored microscope slides are housed in envelopes individually at the Natural History Museum, London in order to protect them from dust and physical damage, in the past in envelopes made of manila, currently in envelopes made of archival-quality polyester (Eastop 1985; Brown 1997). Nowadays, only type material seems to be housed in envelopes. Mounting the envelopes and inscribing them with the specimen data is quite time-consuming (Brown 1997). There is also a risk of damaging a slide while placing it into the envelope. Also, every

profiling requires the profiler to take the slide out of its envelope and put it in again. Wagstaffe & Fidler (1955, pp. 198–199), Hooper (1986b), and Brown & Boise (2005, 2006) suggested glueing a piece of cardboard with the label thicker than the mount left and right of the coverslip in order to physically protect the mount centrally on the slide (for drawbacks see chapter 3.9 Labels). Oosten (2002a) emphasized that plastic degradation is generally accelerated in microclimates. The senior author of this paper is not fully convinced that the advantages of envelopes outbalance their disadvantages. For horizontally stored slides such measures are not regarded as necessary at all.

The Senckenberg Forschungsinstitut und Naturmuseum Frankfurt stores microscope slides of the collection of comparative histology horizontally on cardboard trays either in custom-made wooden cabinets or in cabinets with drawers made for pharmacies (Gudo *et al.* 2012). Some of the cardboard trays include a cardboard top, because several trays are stored on top of each other. This system of storage makes access to the collection more difficult, but the collection is not used for research including loans.

In the curatorships Crustacea, Marine Invertebrata, and "Vermes"of the Museum für Naturkunde Berlin, microscope slides are stored horizontally on open cardboard trays in a more recent and five old custom-made wooden cabinets, each with 80 drawers (Fig. 1A–C). One drawer takes three or four cardboard trays depending on to what extent the trays bend in their central area. The cardboard trays are not of archival-quality yet, and one cabinet is made of oak, so there is potential for improvements.

Less recommendable is storage of microscope slides in boxes holding commonly 25, 50, or 100 slides in a box, but this is sometimes suggested (Wagstaffe & Fidler 1955, p. 197; Martin 1978; Heikinheimo 1988). Although this is a quick way of building up a collection because of the easy availability of the boxes, such a box is not very safe against opening it from the wrong side leaving a mess of slides, and probably also broken slides, on the table if not on the floor (Goodway 1995; Brown 1997, 1998; Gütebier 2011). Also, each box should stand upright on end in order to allow horizontal storage of preparations (Wagstaffe & Fidler 1955, p. 197; Martin 1978; Brown 1998; Gütebier 2011). Profiling of slides stored in boxes is especially impractical, because every box has to be opened and every slide has to be taken out and inspected individually.

3.2.5 Taxonomic arrangement

In the entomological collection of the Natural History Museum in London, vertically stored microscope slides are arranged by supraordinated taxon and then alphabetically by family, genus, and species thus replacing a separate index card catalogue (Eastop 1985; Brown 1997). A similar approach was taken by Heikinheimo (1988). In the curatorships of the marine invertebrates and "worms" of the Museum für Naturkunde Berlin, horizontally stored slides are arranged by supraordinated taxon and then by catalogue number. The latter system allows the curator to ignore systematic changes over time but requires cataloguing of the specimens.

3.2.6 Multiple-specimen slides

Particularly acarologists, nematologists, and parasitologists show a tendency to mount several individuals on a single microscope slide (e.g., Essig 1948; Hooper 1970, 1986a; Eastop & Emden 1972; Jeppson *et al.* 1975, p. 392; Heikinheimo 1988; Amrine & Manson 1996). This practice is advocated with arguments like "taxonomic skills are enhanced by recognizing the presence of more than one species or form on a slide", "difficulty of keeping [...] mites centered, of finding them on the finished slide, the possibility that the quality of clearing and orientation of the mites may be less than desired" (Amrine & Manson 1996, p. 393). Jeppson et al (1975, p. 392) claimed that multiple specimens on a slide "will sharpen the perception of the taxonomist" to segregate different species in a mount. However, multiple specimens on a single slide represent a major disaster from a curatorial point of view. The specimens may not belong to the same species and, therefore, require different catalogue numbers. But often, little space is left for a label or placing the catalogue number(s) with a diamond-tipped engraving scribe on a slide (Fig. 3G). Sometimes, a type specimen is indicated on a multiple-specimen slide with a "permanent" marker, which may get lost over time (comp. also chapter 3.9 Labels). Therefore, the senior author can only strongly recommend mounting only a single specimen on each slide (see also Mound & Pitkin 1972) or even mounting the parts of one specimen on several slides (Bretfeld 1991).

FIGURE 1. Storage of slide collections at the Museum für Naturkunde Berlin (**A**–**E**) and National Museum of Natural History, Washington, D.C. (**F**–**I**). **A**–**D.** Wooden cabinets with drawers and cardboard trays for horizontal storage. Note replacement label for slide on loan (**D**) and slide mounts of Aphidina between two coverslips previously stored with insect on needle (**E**, arrows mark holes from needle in cardboard envelopes). **F**–**I.** Metal cabinets composed of several units by Technicon (**F**–**H**) and Fisherbrand™ (**I**). **G.** Metal tray with holes (arrows) for easy access to standard slides from below. Arrowheads mark bent-up slide holders. **H.** Frontal view of opened single unit. **I.** Dense vertical storage of slides of similar size. Photographs **F**–**I** courtesy of Cheryl Bright. **D**–**F**: macro lens.

TABLE 2. Worksheet for profiling a slide collection and documenting subsequently achieved goals (fields marked in grey).

Curation status of slides in collection .....: Profiling & Correction Worksheet

Cabinet No.: **Taxon**: recorded by: Date:
Cabinet (ok = ü; defective = circle)**:** *wooden (oak / other) / metal cabinet lock hinge dust_protection / rubber_liners drawers / guide_rails slide_trays_plane*

drawer/ taxon	**slide tray**	total number of slides	number of catalogue numbers	**level 1: danger of loss of primary information**				**slide physically ok / restored**			
				a – label: missing / fading / loose, 'permanent' marker on glass, catalogue number not engraved with diamond scribe	**b** – slide: broken / wood / plastic / cardboard	**c** – coverslip: none / loose; sections not dyed	**d** – mounting medium: crystallization / cracks / cavities / blackening	**level 2:** unidentified; not catalogued	**catalogued**		
									level 3: identified to suprataxon	**level 4:** identified to family / genus level	**(level 8):** identified to species level
	1 (= top)										
	2										
	3										
	4										

Remarks: number of **histological slides** (= slides suitable for digitalization): ; Cobb Aluminum;
size of slides: standard, excess width, excess length, oversized, short

Mouth parts and legs of arthropods are often dissected and mounted on a microscope slide. Sometimes, each body part is covered with a small circular coverslip (Martin 1978, p. 157; Noyes 1982, fig. 9) instead of covering the entire ensemble with one large coverslip, which is less prone to damage at its edges than several small coverslips (Fig. 2C, E). One large coverslip is also easier to clean than multiple small ones. The problem of mounting several body parts while keeping a favored orientation can be easily overcome by applying a small amount of mounting medium, orientate the body parts as preferred, let this dry for some time, and finally apply the coverslip with some fresh mounting medium (Moseley 1943a; Hamond 1969; Tagestad 1976; Noyes 1982; Koomen & Vaupel Klein 1995). In this way, the body parts will stick to the basal medium and will not move anymore. Body parts may also be fixed to the coverslip in the ways described previously and later mounted on the glass slide by inverting it in a drop of Canada balsam. In this way, the specimen is closest to the coverslip, and the mount offers best resolution (Moseley 1943a). Monk (1938) suggested using a mixture of dextrose (Karo corn syrup) and fruit pectin (Certo) to fix body parts to the glass slide before mounting in Euparal, but this solution may lead to conservation problems over time because of incompatible chemicals.

3.3 Collection management

3.3.1 Profiling of slide collection

The senior author of this paper strongly recommends inspecting microscope slide collections regularly (Hooper 1986b) by profiling (Tab. 2) as should be done with every other storage of natural history specimens be it in a wet or in a dry collection. A profiling interval of about 10–15 years is suggested, control of specimens mounted in glycerol may be conducted about every 5 years (Tab. 14). These intervals are not based on scientific criteria yet but may allow recognition of upcoming damage early enough to be able to react properly to a problem.

The Smithsonian Collections Standards and Profiling System offers a detailed system of 10 levels for the evaluation of the curation status of natural history collections, viz, material conservation (level 1), specimen accessibility (levels 2–4), physical organization (levels 5–6), data capture (levels 7–9), and scientific voucher material (level 10) (McGinley 1989: pp. 20–21, 1992, p. 311). Especially the higher curation levels require in the senior author's opinion considerable familiarity both with collection management and with the taxonomy of the respective group. Also, the original suggestions concentrate on assessment units such as insect boxes. In this article, a slightly different approach is presented for the assessment of slide collections (Tab. 2). We focus more on those aspects, which can be observed directly on the slides by students after a limited time of training. Aspects like discoloration of histological sections in the periphery of a slide are not a subject of the profiling, because this situation cannot always be recognized satisfyingly at a glance and the problem may not be overcome in a straightforward approach. In addition, a slide collection is evaluated on the basis of individual slides. Otherwise, the amount of work would be grossly exaggerated, because certainly every tray of about 40–50 slides will contain level-1 slides. The worksheet suggested here also offers the advantage that achieved goals like restoration of slides can be entered in the form sheet in the fields marked in grey at any time (Tab. 2).

Generally, a recording person will assess the microscope slides with the help of a worksheet offering various physical and curation conditions of a cabinet, tray, and slide (Tab. 2). The most alarming level 1 (= danger of loss of primary information) is recorded most intensely, because here the need for action is most urgent, and the time required for improving the situation is especially high. Curation levels 2–4 and to some degree 8 can also be gathered directly from a slide by checking for a catalogue number and for the identification to different taxonomic levels (Tab. 2). Usually, original labels of slides are not removed, and new labels or new determination results cannot be added on an existing label because of limited space on the slide. Therefore, curation levels 5 and 6 are often not reached on a slide itself but in the catalogue and database. Curation levels 7–10 can only be accessed from the written catalogues or from the collection database but not from the microscope slide itself. Therefore, these curation levels are not part of the assessment worksheet.

3.3.2 Loan of slides

Microscope slides from museum collections are often given on loan to other scientists. The slides should be padded

with cotton wool, soft laboratory tissue, or bubble wrap in order to avoid rattling in the slide box. In addition, the entire box should be well-padded and sent in a larger parcel (Martin 1978, p. 121; Pritchard & Kruse 1982; Hooper 1986b). If slides are shipped in a hinged cardboard tray for one, two, or several slides, a rubber band around the slide at each label will prevent the coverslip from touching the cardboard surface and moving if the medium has not dried sufficiently (Amrine & Manson 1996, p. 395). Top and bottom sides of such cardboard trays should also be closed by a piece of tape and the top side indicated in order to avoid broken slides during unpacking. The loan form sheet plus proper declaration (*inter alia* the popular name of the taxon mailed and a statement that the material represents a museum specimen, has been initially fixed in formaldehyde, is not infective, and is of no commercial value) should be both included inside the parcel as well as attached outside for customs inspection. This may be self-evident, but experience teaches us it is not, thus customs inspection and subsequent veterinary inspection will delay delivery of a parcel and cost extra money for the unnecessary veterinary inspection (Neuhaus pers. obs.). The senior author suggests indicating the missing slides on the tray of the sending collection with a piece of paper noting the specimen's name, catalogue number, borrower, and the date of loan (Fig. 1D). Especially careful staff may want to photograph each slide before it leaves the museum for a loan in order to have some documentation of the original label in case the slides gets lost during the loan. Alternatively, entire trays with horizontally stored slides could be photographed as a prevention or even the entire slide collection, but this would require a considerable amount of time—and the question becomes how much time is one willing (or allowed) to devote to such tasks, while the slides with the original specimens may be deteriorating.

Scientists receiving specimens on loan may want to make microscope slide mounts from material previously stored in ethanol. The senior author suggests that the lending institution insists on mounting the specimens in a medium, which is known either to last for many decades such as neutral Canada balsam and Euparal or to be reversible like glycerol-paraffin mounts. If slow-drying media like Canada balsam and Euparal are used, the freshly made microscope slides must be allowed to dry entirely before shipping, preferably in an oven or on a hotplate (Brown 1997; Amrine & Manson 1996; Neuhaus pers. obs.); otherwise the coverslip may move, and the mount be spoiled. Plastic strips as are often used for mounts of insect genitalia must be avoided under all circumstances. With the help of these general guidelines, the situation would improve at least for future microscope slides in a collection.

Recently, the Museum für Naturkunde Berlin established in the frame of a pest management program a quarantine and freezing station for incoming parcels with special attention to entomological items returned from the borrower. Material from wet collections and microscope slides are excluded from this practice, because both mounting media (Brown 1997, p. 6) and coverslip seals containing polymers (Shashoua 2008, p. 203) would suffer from shrinkage or formation of large hexagonal ice crystals, which pierce cell membranes and the like during the freezing process (Florian 1990; Allington & Sherlock 2007a, 2007b). The latter authors demonstrated that damage occurred already after freezing coverslip seals just a few times. The consequences of these processes have already been recognized at the Natural History Museum in London (Brown 1997).

3.3.3 Cleaning of slides

Microscope slides require cleaning depending on how well the cabinet protects against dust and how intensely the collection is used. Dust, glass particles abraded from specimen slides, flakes of the microscopist's skin, fibers from clothing, pollen, fingerprints, grease, remnants of immersion oil, and the like (Zölffel 2011) can be removed with a rubber dust blower or a fine brush; subsequently, a dry disposable soft lab tissues can be used or a tissue wetted with distilled water and in more persistent cases with ethanol without drowning the slide in the liquid (for more details see also chapter 3.12.2 Optical surfaces, immersion oil; Gütebier 2011; Gudo *et al.* 2012). Garner & Horie (1984) favored Draft-Clean, a powdered art rubber, for cleaning slides of grime. Also, absorbent purified cotton wool rolled on a skewer (Fig. 27A) can be applied successfully (see also chapter 3.13.2 Optical surfaces, immersion oil; Zölffel 2011). Immersion oil is best removed first with a disposable lab tissue and subsequently with ophthalmic cotton wool and ethanol (Kämpf pers. obs.). Gütebier (2011) advocated handling of slides exclusively with a pair of forceps enwrapped at its flat tips with protective adhesive tape; this seems to be a bit impractical in everyday scientific work. He also recommended not to wipe slide surfaces, but only to roll wetted cotton wool over slides. In the senior author's experience this is often not sufficient to remove the dirt, especially if cleaning has not been done for an extended period of time.

3.3.4 Documentation and curation

Mounting medium and coverslip seal used for a specific specimen should be documented in the registry catalogue/database (Tab. 14) but often are not. This makes later restoration efforts tedious. In a separate paper, we address the use of a modern non-destructive method for identifying unknown mounting media and coverslip seals, which would significantly simplify the correct choice of restoration technique (Schmid *et al.* 2016; comp. chapter 3.10 Restoration procedures).

Cushing (2011) estimated that curation of a collection of 10,000–20,000 microscope slides required at least one half-time position of permanent staff plus a part-time student assistant.

3.4 Slides

3.4.1 History of slides

In the late 17th century, small specimens were usually observed mounted on vertically oriented sliders made of ivory or hardwood. Each slider contained a series of holes, in which the specimens were placed between two pieces of mica, a sheet silicate consisting of the mineral muscovite. The slider was moved from one hole to the next hole, hence the name slider and its successor, the slide (Tab. 1; Smith 1915; Bracegirdle 1978, p. 18, text-fig. 4, color plate 1; Gill 2013, p. 42). Later, the ivory and hardwood were replaced by a strip of glass with or without a coverslip made of mica or thin glass in order to reduce evaporation of liquid mounts. Gütebier (2011) claimed that until the middle of the 19th century coverslips were prepared from selenite, a derivate of gypsum. The first glass coverslip was introduced in 1789 by Ingen Housz, but common use mainly for fluid mounts was not before about 1835. It seems that coverslips were applied to turpentine-cleared specimens mounted in balsam at and after 1843 (Tab. 1; Bracegirdle 1978, pp. 23, 113–114). At this time, microscope slides of various sizes were used and studied now in a horizontal orientation, and it was not before 1839 that the provisional committee of the later Royal Microscopical Society of London fixed the size of glass microscope slides for its own collections to 3 inches by 1 and to 3 inches by 1.5 (Tab. 1; Bracegirdle 1978, pp. 111–112; Gill 2013, p. 42). These sizes became quickly the standard in England, probably also because of commercial interests of mounting companies. Nevertheless, several different sizes continued to exist in Europe until about the late 1880s (Smith 1915; Bracegirdle 1978). Extra-broad slides are still used nowadays for large histological sections.

3.4.2 Variety of slides

Nowadays, a considerable variety of microscope slides can be met beside the size of the standard glass slide (76 mm x 26 mm = 3 inch x 1 inch) in larger museums. Slides may vary in length, width, and thickness, e.g., 75.5 mm x 26 mm x 3.6 mm, 48.5 mm x 27.4 mm x 2 mm (= new Gießen format), 88 mm x 45.2 mm x 1.2 mm (Figs 2A–H, 3A–G; Bracegirdle 1978, p. 112). Thick slides may lead to problems with correct Köhler illumination (see chapter 3.12 Study of specimens and documentation). Historical, rather unusual slides have been mounted in the 19th century on irregularly shaped pieces of glass and painted in the worm collection at Berlin (Fig. 2H) or as double-coverslips on a thicker cardboard (Fig. 4G, H) or between two fused thin pieces of cardboard (Fig. 4F). Especially the latter microscope slides and short slides (Fig. 3A–E) are difficult to handle on a modern microscope table with its spring holder, so a larger aluminium supporting slide is needed for study. Home-made two-coverslip mounts fastened with paper strips or labels in the worm collection at Berlin include also frames made of wood (Fig. 4I) or plastic (Fig. 4D, E). These types of slides are not very useful, the former because the wooden frame is very thick and problematic to get correct Köhler illumination, the former and latter because the coverslip detaches easily from the frame or may bend or distort over time (Fig. 4C, E, H; see also Higgins-Shirayama slides in chapter 3.4.4 Double coverslip mounts). Humes & Gooding (1964) suggested using softwood with a central hole of 15 mm diameter as slide and imprint a rim of 4 mm with a metal stamp. The larger coverslip with a 22 mm diameter was supposed to be fixed with glue or nail varnish. In contrast to the Cobb aluminium slide and the Higgins-Shirayama slide, the technique of Humes & Gooding (1964) requires the second coverslip to be mounted on the reverse side of

the slide. The disadvantage of this method is that less lateral space is available for manipulating the smaller coverslip. Wooden slides cannot be recommended here for permanent slides at all because of the inherent deterioration processes in the wood (see discussion of cabinets in chapter 3.2 Storage).

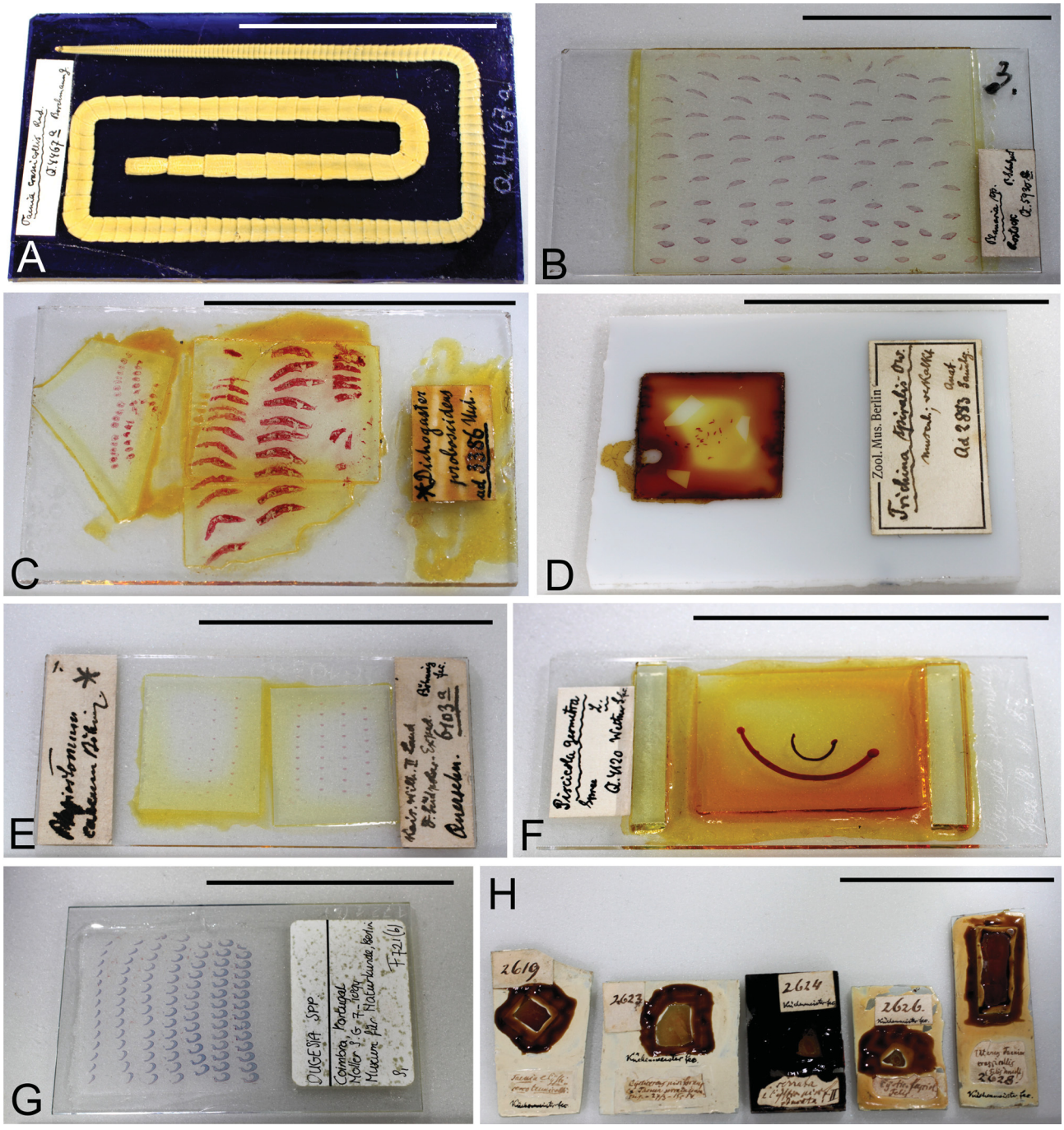

FIGURE 2. A, D. Cestode (**A**) and nematodes (**D**) on oversized blue and white-opaque glass plates, respectively; before 1901 by Borchmann (**A**) and 19th century by an unknown person (**D**). Specimen in **A** without coverslip. **B, C, E, G.** Histological sections of flatworms (**B**, **E**, **G**) and oligochaete (**C**) on oversized glass plates; before 1927 by Schulze (**B**), before 1897 by Michaelsen (**C**), before 1914 by Böhmig (**E**), and in the 1980s in DPX in the lab of Sluys (**G**). Label in **C** glued and covered with mounting medium. Self-adhesive label in **G** with glue having migrated through the label indicated by dark spots on the surface of the label. **F.** Hirudinea on large glass plate with top glass bars; before 1917 by Weltner. Notice yellowed mounting medium in periphery of coverslips in **B**–**F**. **H.** Cestodes on painted pieces of glass; middle of the 19th century by Küchenmeister. **A**–**H**: macro lens**.** Scalebars: **A**–**H**, 5 cm.

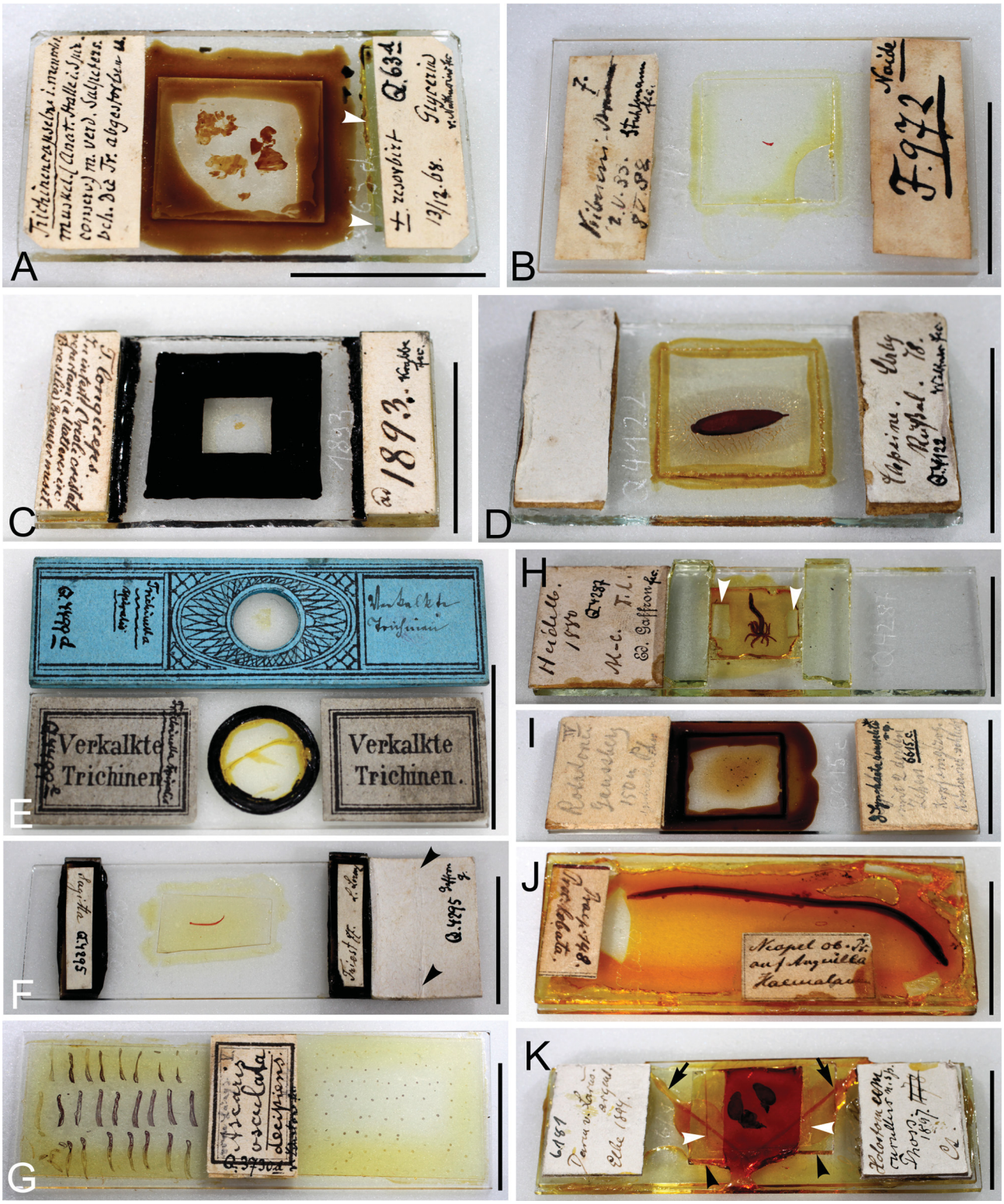

FIGURE 3. Small-sized (**A-E**) or otherwise unusual slides (**F-K**). Notice bottom (**A**, arrowheads) or top glass bars (**C**, **F**, **H**), thick cardboard labels (**D**, **G**, **I**, **K**), label extending beyond glass slide (**F**, arrowheads mark end of slide), yellowed mounting medium in periphery of coverslips (**G**), and slide with top glass slide instead of coverslip (**J**). Coverslip mounted on pieces of glass as spacer (**H**, arrowheads). **K.** Trematode mounted between two glass bars (black arrowheads) under coverslip (white arrowheads mark coverslip margins); broken glass slide repaired with the help of a 2nd lower glass slide, cracks of upper slide indicated by black arrows. Specimens mounted in glycerol (now dry) 1868 by von Nathusius (**A**), in unknown media1888 by Stuhlmann (**B**), in the 19th century by Krabbe (**C**), before 1917 by Weltner (**D**), before 1918 by an unknown person (**F**), before 1914 by von Linstow (**G**), before 1918 by Gaffron (**H**), between 1904 and 1927 by Zelinka (**I**), between 1906 and 1937 by Wilhelmi (**J**), and before 1897 by Thoss (**K**). **A-K**: macro lens. Scalebars: **A-K**, 2 cm.

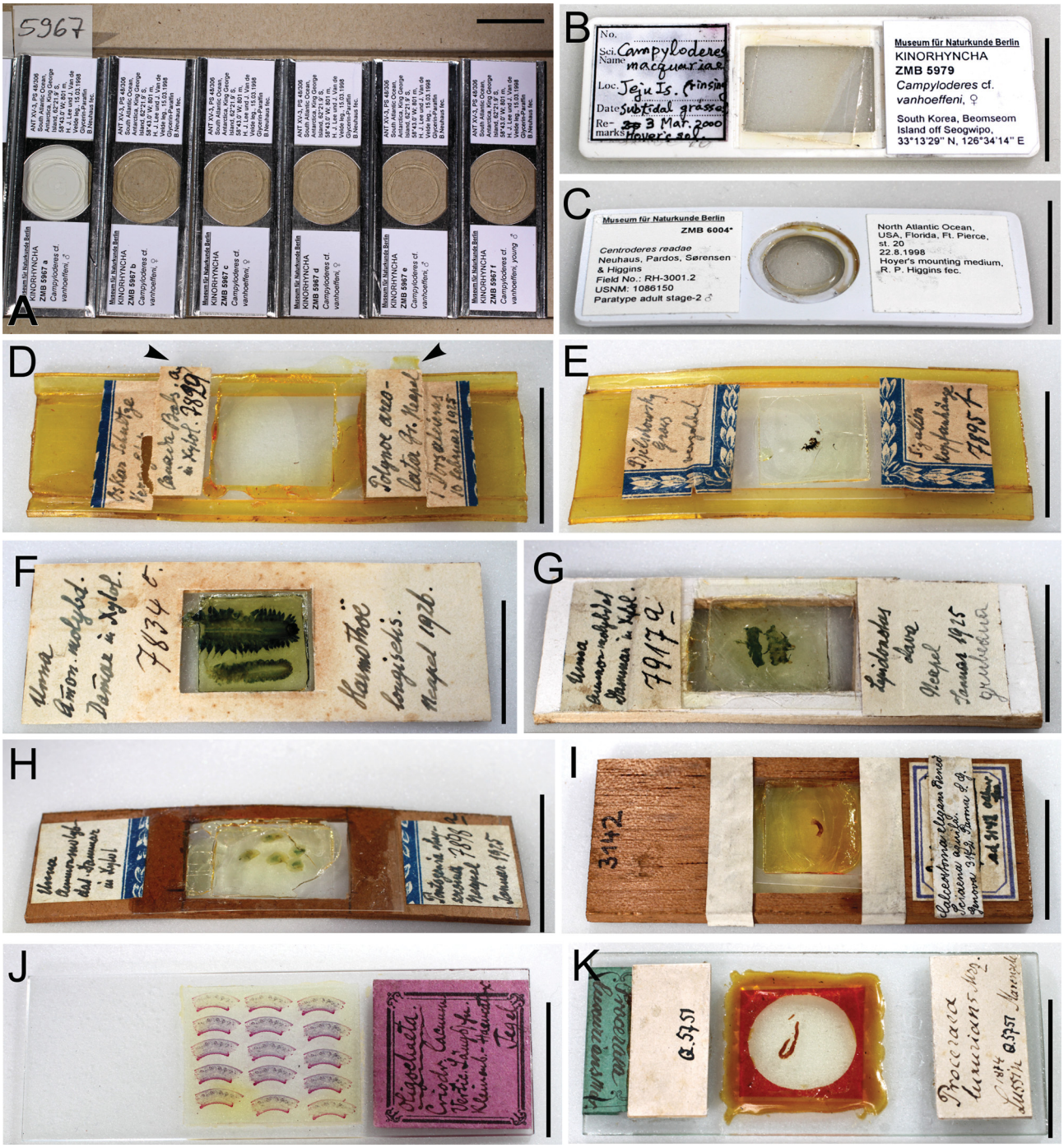

FIGURE 4. A-I. Double-coverslip slides with frames of different materials: aluminum (**A**, Cobb slides), plastic (**B**, **C**: Higgins-Shirayama slide with rectangular opening in **B** and circular opening in **C**; **D**, **E**: aged plastic, coverslip held in place by label paper and detached in **D**, see arrowheads), thin (**F**) or thick (**G**, **H**) cardboard, and wood (**I**, coverslip held in place by paper strips). Notice that certain types of plastic and cardboard slides bend upwards centrally (**C**, **E**, **H**) or get easily distorted (**D**). **J.** Stains faded and unknown mounting medium yellowed in the periphery of the coverslip. **K.** Slide with cardboard labels and spacer consisting possibly of coloured paper tissue. Mounted as glycerol-paraffin mounts between 1999 and 2010 by Neuhaus (**A**), in Hoyer's medium between 2000 and 2001 by Song and Chang (**B**) and between 1998 and 2004 by Higgins (**C**), in Canada balsam after 1925 by Schultze or Heider (**D**, probably **E**), in gum dammar dissolved in xylene after 1925 by Heider (**F-H**), and between 1874 and 1890 by Marenzeller (**K**). **A-K**: macro lens. Scalebars: **A-K**, 2 cm.

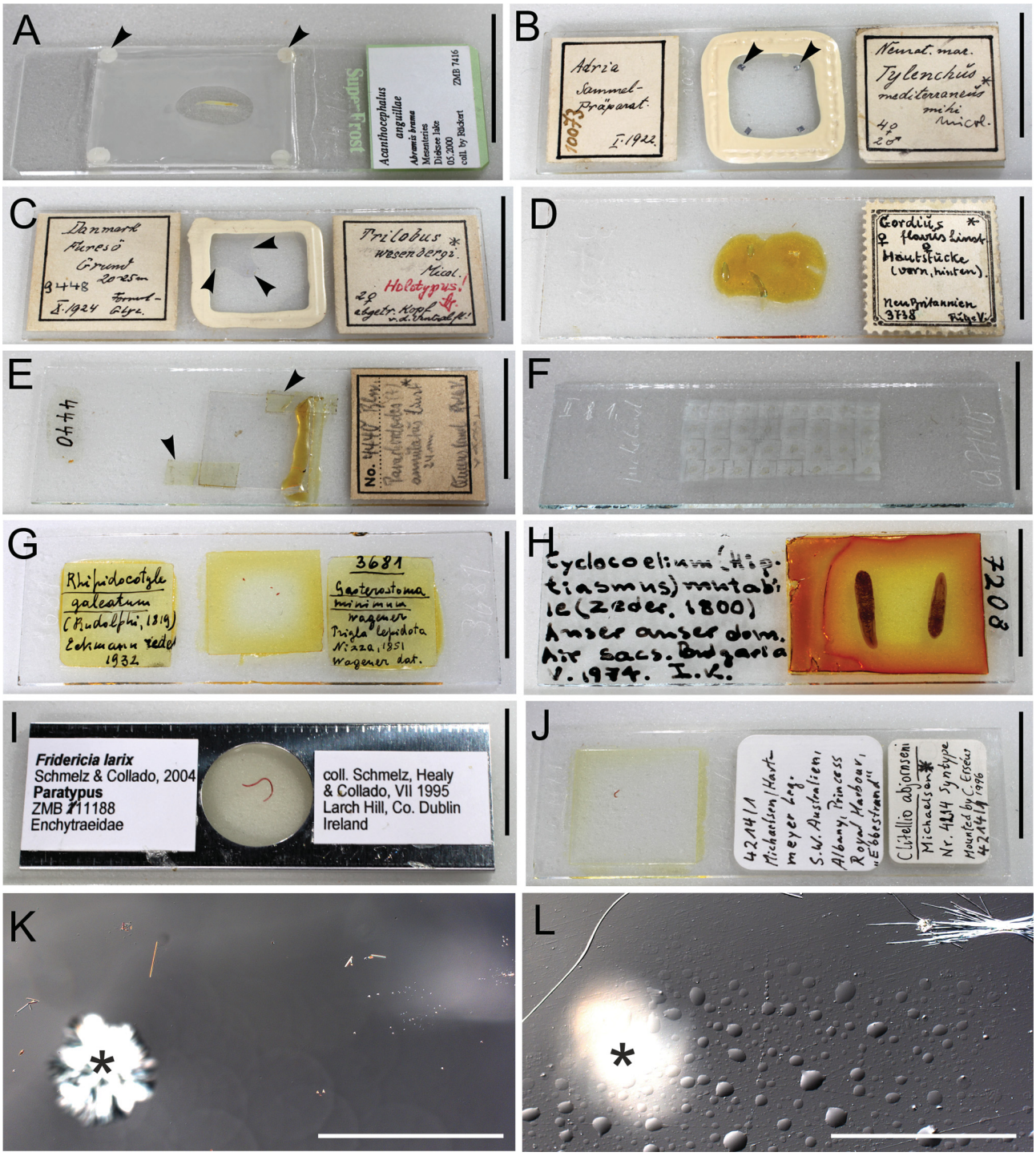

FIGURE 5. A. Glycerol-paraffin mount with spacers (arrowheads), probably some kind of modelling clay; made between 2000 and 2007 by Rückert. **B, C.** Glycerol mounts with metal spacers (**B**, arrowheads) and cavities because of evaporation of glycerol (**C**, arrowheads), sealed with a white varnish; early 1920s by Micoletzky. **D, E.** Pieces of epidermis and cuticle of nematomorphs; early 1930s by Heinze. Notice lack of coverslip (**D**) and coverslip fastened to slide with adhesive tape (**E**, arrowheads) and a small amount of a mounting medium. **F.** Unfinished histological sections still in paraffin, unstained, and without coverslip; early 1920s by Arndt. **G.** Specimen information inked on glass slide and covered with mounting medium and coverslips; before 1932 by Eckmann. **H.** Specimen information written with a "permanent" marker on glass side, partly wiped off during cleaning of slide with lab tissue; after 1974 by Kanev. **I, J.** Slides with self-adhesive labels; glued on back side of slide, and coverslips adhered with mounting medium to aluminum frame (**I**); mounted in Malinol between 1995 and 2004 by Schmelz (**I**) and in an unknown medium in 1996 by Erséus (**J**). **K, L.** Surface of dry borosilicate coverslip (**K**) and of wet soda-lime glass slide (**L**) of same slide at same spot (note white crystal in mounting medium marked by asterisk). **A-J**: macro lens; **K**, **L**: DIC. Scalebars: **A-J**, 2 cm; **K, L,** 500 µm.

Older slides often reveal a pair of thick glass bars on top (Figs 2F, 3C, F, H) or below the slide (Fig. 3A) or a thick cardboard label (Fig. 3D, G, I, K). Possibly, these structures served to protect the coverslip and mount if slides were stacked on one another.

Polystyrene slides like Permanox™ can only be used with water-based mounting media and will warp if exposed to organic solvents for an extended period of time. Such slides cannot be recommended for mounts of taxonomic importance, because the polystyrene will degrade inevitably and much quicker than glass slides.

In some entomological collections of the Museum für Naturkunde Berlin it has been practice to mount the genitalia of a specimen between a square basal and a round top coverslip, to protect the coverslip mount with a rectangular thin cardboard sleeve, and to pin this ensemble with a needle below the body of the specimen (see also Brown 1997, p. 4). These microscope mounts have been removed from the insect boxes and stored in a regular cabinet for microscope slides for quite some time now (Fig. 1E). In other collections, the genitalia of a specimen are still mounted with Canada balsam or Euparal covered by a small piece of a coverslip on a narrow strip of plastic and attach the strip to the needle together with the insect and the labels. The genitalia on the strip are occasionally re-mounted by dissolution of the medium in xylene and re-mounting in the medium if a different orientation of the genitalia is necessary for examination (Jäger pers. com.). A strip of celluloid holding a mount of insect body parts between two coverslips has been known for a long time (Imms 1929, pp. 166–167; Martin 1978, pp. 104–105). Celluloid is a cellulose nitrate polymer with the volatile plasticizer camphor (for conservational aspects see chapter 3.8.9 Nail varnish). Plastic strips seem to have been introduced in German-speaking countries in the 1970s (Jäger pers. com.) and consist originally of cellulose acetate; trade names are Ultraphan in Germany and Acetat in Switzerland (Besuchet 1974, p. 306). This cellulose acetate seems to possess a degree of substituted hydroxyl groups by acetate at an average of two. Cellulose acetate oxidizes at room temperature and becomes more brittle over time (Horie 2011, p. 213). Cellulose acetate strips used by C. Besuchet in the Coleoptera collection of the Museum für Naturkunde Berlin last at least 40 years (Jäger pers. obs.). Other kinds of plastic strips in the Coleoptera collection became brittle or twisted considerably over time. These developments make handling and observation of the mounts extremely difficult if not impossible. Probably for these reasons, this method has been rejected earlier (Robinson 1976) and has now been abandoned at the Natural History Museum in London (Brown 1997, p. 4, fig. 10).

3.4.3 Glass slides

Standard glass microscope slides should comply with DIN ISO Norm 8037/I, should have a size of 76 mm x 26 mm at a thickness of 1–1.2 mm, and are usually manufactured from hydrolytic class 3 soda-lime glass. These slides possess a low iron content and are cleaned, extra white, and free of surface defects, inclusions, streaks, and bubbles (e.g., Menzel-Gläser, Microcrown glass for microscope slides) (Tabs 12, 14). Older slides in museum collections often consist of a considerably lower quality of glass with streaks and bubbles and revealing a greenish color if seen from the side. The greenish color originates from a mixture of ferrous oxide (FeO) and ferric oxide (Fe_2O_3) in the glass (Davison 2003, p. 6), which is indicative of a low glass quality with a reduced light transmittance (Al-azzawi 2013). It is recommended to use slides with ground edges in order to reduce the danger of cuts to the fingers during handling and abrasion on the microscope stage (Wagstaffe and Fidler 1955, p. 193).

The surface of the soda-lime glass is hygroscopic and always coated by water many molecule layers thick (Davison 2003, p. 206). In the working room of the senior author with regular aeration, the surface of soda-lime glass slides stored in a wooden cabinet may be covered by visible drops of liquid (Fig. 5L), whereas the borosilicate glass of a coverslip on the same slides and slides made from poly(vinyl chloride) do not reveal visible drops of liquid (Fig. 5K), which is in accordance with experimental data for glasses of different composition (Adams 1984, fig. 15). This phenomenon has not been observed in the slightly colder and dryer storage area of the slide collection. Generally, soda-lime glass decays over time, both through corrosion by liquid substances and through weathering by atmospheric gases and vapors, because sodium and potassium cations of the respective oxides in the glass are able to freely move within the glass network (Davison 2003, pp. 3–6, 173–176; Melcher *et al.* 2010). The cations react with moisture from the air or with water to sodium hydroxide resulting in leaching of the surface and increasing alkalization, which will, above a pH of 9, finally lead to a breakdown of the silicon-oxygen bonds and consequently of the glass network (Newton 1985; Davison 2003, p. 175; Melcher *et al.* 2010).

Weathering may happen via sodium hydroxide reacting with carbon dioxide to sodium carbonate, which deposits as a crust on the glass surface (Davison 2003, p. 190; Sturm 2006). These chemical transformations may occur already at 20% relative humidity (Sturm 2006). The leached surface of glass has been termed *inter alia* alkali-deficient layer, silica-rich layer, or hydrated gel layer (Newton 1985; Davison 2003, pp. 174, 195–196; Melcher *et al.* 2010). Borosilicate glass, which contains significantly less sodium oxide than soda-lime glass, deteriorates at a considerably slower rate and loses about 100x less substance in comparison to soda-lime glass in water at 25°C within the same time interval (Adams 1984, fig. 16, tab. 2; Newton 1985, fig. 12, p. 33). Therefore, Gütebier (2011) suggested using slides made of borosilicate glass, but these are mainly available coated for DNA microarrays (e.g., Conzone & Pantano 2004) and would have to be custom-made without coating. He also stated that slides made from soda-lime glass at the Gothenburg museum, Sweden, deteriorated significantly and showed dissolution of the glass components in high relative humidity; he also illustrated this situation (Gütebier 2011, p. 71, fig. 5). To the senior author, his figure 5 does not show deterioration of the glass surface but deterioration of the mounting medium with typical cracks and cavities. There is dirt on the glass surface, which appears overly expressed, because the condenser aperture seems to have been closed to a degree that diffraction artifacts become visible. In the collection of the Museum für Naturkunde Berlin, even old microscope slides do not show obvious signs of glass decay like a milky or opaque, rough, iridescent surface, which should be easily recognizable based on the senior author's experience from glass jars in our wet collection (Neuhaus *et al.* 2012). The absence of unmistakable signs of degradation of the glass surface of slides may either result from acidic or neutral attack by humidity and acids from the deterioration of wooden cabinets, adhesives, and paints which, in contrast to alkaline attack, does not produce cracks and flaking on the glass surface (Melcher *et al.* 2010). Alternatively, notable glass degradation of microscope slides may represent more of a problem under humid conditions and tropical temperatures but less so in temperate zones under museum conditions. For this reason, slides designed for applications in the tropics are sealed in evacuated aluminum-coated plastic bags and a paper liner between adjacent slides. Remarkably, acids and aldehydes originating from the degradation of wooden cabinets in a Scottish museum lead, together with fluctuating temperature of 12–25°C and relative humidity of 20–65%, to the deterioration of glass artifacts (Robinet *et al.* 2004). It remains open to what degree microscope slides decay in temperate climates.

Depression slide. With the help of a spherical carborundum tool, Gisin (1968) drilled a depression (3.5 mm diameter x 0.2 mm depth, up to 5.5 mm diameter x 0.4 mm depth) into a glass slide of 0.6 mm thickness to accommodate a small Collembola in lactic acid; the coverslip is sealed with a mixture of paraffin and lanolin, also including or not white vaseline. Slides with depressions of different depths are commercially available for quite some time now (Wagstaffe & Fidler 1955, p. 194), so there is no need for home-made solutions anymore. In fact, depression slides have been used for mounting rotifers already in the 19th century (Rousselet 1895, p. 11).

3.4.4 Double-coverslip mounts

The problem of insufficient observation of a specimen's reverse side has been known for a long time and was overcome with double-coverslip mounts, where two coverslips sandwich the specimen. This arrangement allows the user to flip over the microscope slide and to observe a specimen also from the reverse side. In addition, at least modern coverslips are made of borosilicate glass, which is much more resistant to weathering and corrosion than soda-lime glass (see chapter 3.6 Coverslips).

Cobb aluminum slide. One of the oldest double-coverslip mounts represents the so-called Cobb aluminum slide (Tab. 1; Cobb 1917) in which the specimen is sandwiched between a 24 mm x 24 mm square bottom coverslip and a circular, 15 mm diameter top coverslip and mounted between two pieces of medium-thickness cardboard in an aluminum frame (Figs 4A, 27C, D; Cobb 1917; Hooper 1970, 1986a; Higgins 1971, 1988). If the lateral edges of the aluminum frame are not pressed flat on the two pieces of cardboard, the slide may not be held safely by the slide holder on the microscope's stage and may be flipped from the stage (Hooper 1986b; Neuhaus pers. obs.). This problem can be overcome by inverting the aluminum frame during mounting and pressing the edge twice, once from the top side and once from the bottom side (Fig. 27D). The slide can be easily dismantled and re-mounted, e.g. for restoring processes, because the larger coverslip is only mechanically held in place by the aluminum frame and two pieces of cardboard. The Cobb slide is strongly recommended for the light microscope study of total mounts of meiofauna and other small invertebrates (see also Hooper 1986b; Bartsch 1988). A further advantage is

that the aluminum of the slide frame does not corrode significantly once a layer of aluminum oxide has built and protects deeper layers of aluminum against corrosion by water and air pollutants, so this material allows long-time storage in museum collections (Tab. 14). The Cobb aluminum slide should be complemented with archive-quality labelling paper and medium-thickness cardboard according to DIN/ISO 9706 and archive-quality scripting (see chapter 3.9 Labels; Neuhaus 2013). If the cardboard is too thick, high-power objectives may bump onto the cardboard, deliver part of the immersion oil to the label, and partly dissolve the inscription (Hooper 1986b; Neuhaus pers. obs.). For this reason, the central aluminum edges opposite the coverslip are pinched quite flat and the basal coverslip is 32 mm long rather than 24 mm in order "to give more lateral clearance for objectives" at the Nematology department of the Rothamsted Experimental Station (Hooper, 1986b, p. 316). No general manufacturer of the raw aluminum frames for Cobb slides is known currently, so the frames must either be home-made (Courtney 1936; Jutras & Tarjan 1961; Mason & Bosher 1963) or produced by a local metalworking company.

Higgins-Shirayama slide (H-S slide). More recently, the Higgins-Shirayama slide (H-S slide) slide was developed by R. P. Higgins and Y. Shirayama. This microscope slide possesses the size of a standard glass slide and consists of two pieces of plastic fused together and revealing a central circular hole, 16 mm in diameter in the lower element and 18 mm diameter in the upper element (Fig. 4C; Westheide & Purschke 1988). Subsequently, Shirayama *et al.* (1993) describe a similar slide but consisting of a single piece of acrylic plastic with a rectangular central hole. A larger hole 23 mm x 25 mm seems to be cut out over a smaller hole16 mm x 22 mm (Fig. 4B). This later variation offers the advantage of using high-power immersion oil-lenses even if the specimen is not located exactly in the center of the hole, because the hole is wider and the front end of the objective does not collide with the edge of the plastic frame. Both variations of the H-S slide can be flipped over and a specimen can be studied from its reverse side. Unfortunately, the plastic H-S slide with the circular hole bends significantly upwards in its central area after several years (Fig. 4C) and becomes extremely difficult to handle on a microscope's table, especially from the reverse side (Neuhaus 2013). We suspect that the bending originates from the fusion (glue?) of the upper and lower plastic frame of the slide. Also, re-mounting for restoring purposes is more difficult, because the larger coverslip is glued to the lower part of the slide. The later variation with the rectangular hole does not seem to bend like the frame with the circular hole, probably because it consists of a single piece of plastic. Generally, this type of microscope slides is not suitable for long-time storage in a museum collection, because any plastic material will inevitably deteriorate after a couple of decades and certainly much quicker than glass.

3.5 Spacers

Spacers allow construction of microchambers for small specimens and even to roll a specimen around its longitudinal axis if mounted in a liquid medium (Lillo *et al.* 2010). Hooper (1986b) strongly advocates extensive application of spacers in order to prevent the coverslip from flattening the specimens (here nematodes) too much if the medium sets over time and if immersion oil objectives are used. However, care should be taken to select archive-quality materials like paraffin wax (see chapter 3.8.11 Paraffin), fiberglass, glass rods drawn out in a Bunsen burner, pieces of a broken coverslip (Fig. 3H, K), or gold wire; only pieces of one and the same coverslip should be used as spacers for a given microscope slide, because different coverslips vary in thickness (Moseley 1943a, 1943b; Newell 1947; Wagstaffe & Fidler 1955, p. 195; Spinell & Loveland 1960; Norris 1961; Hooper 1970, 1986a, 1986b; Martin 1978, p. 142; Gerakaris 1984, p. 262; Huys & Boxshall 1991, p. 452; Hooper *et al.* 2005; Orajay 2005a, 2005b; Jersabek *et al.* 2010; Lillo *et al.* 2010). It remains open whether aluminum wire (Bassett 1941; Sanderson 1994, p. 152) and aluminum foil (Fig. 5B; Stosch 1974, p. 135) may or may not react with a given mounting medium. Transparent fishing lines made of polyamide come in different diameters from 0.1–1 mm thickness and are recommended as spacers for both water- and hydrocarbon-soluble media (Gleiss 1967). The use of nylon in conservation has been discussed controversially (Horie 2011, pp. 194–196). Paper or thin cardboard soaked in soft paraffin (Spence 1940b), in the mounting medium (Fig. 4K; Gatenby & Beams 1950, p. 218; Wagstaffe & Fidler 1955; Martin 1978, p. 106; Brown 1997), or in the coverslip seal (Gray 1954, p. 12), gummed paper (Russel 1950), strips of celluloid (Wagstaffe & Fidler 1955, p. 194; Brown 1997), kapok and wool fibers (Jeppson *et al.* 1975; Lillo *et al.* 2010), modelling clay (Fig. 5A; Webb 1997), and a red rubber ring cemented with goldsize (Ward 1953) certainly do not qualify for long-time storage (for deterioration of plant material see

also chapter 3.2.2 Cabinets), although some of the rubber ring-goldsize mounts may last more than 50 years (Spence 1941b; Ward 1953). Self-adhesive office aid reinforcers suggested by Foulkes (1983) also do not belong into the category of durable spacers, both because of the adhesive and the plastic material. Heating the reinforcers and the chemicals involved in the preparation process twice for eight hours each (Foulkes 1983) does not really represent a proof for stability over decades.

3.6 Coverslips

One of the earliest British companies producing coverslips (= cover glasses) in 1840 is Chance Brothers and Co., Ltd. (Tab. 1; Anonymous 1941; Bracegirdle 1978, p. 113). Originally, coverslips were produced from soda-lime glass, because borosilicate glass was first invented by Otto Schott in the late 19th century. All glass coverslips until about 1928 "would not withstand tropical conditions without becoming cloudy" (Anonymous 1941); this statement may indicate their composition of soda-lime glass, which is considerably less resistant to deterioration under humid conditions. Nowadays, glass coverslips are extra white, highly transparent, colorless, free of blisters and flaws, and made from hydrolytic class 1 borosilicate glass D 263™ (e.g., Menzel-Gläser, Tabs 12, 16). Dimensional tolerances, thickness, optical properties, etc. are standardized by DIN ISO 8255-1 and ASTM Standard E211 (Gill 2013, p. 282; Available from: http://www.menzel.de/files/coverglass-d-263-m-en.pdf, accessed 19 January 2016). The refractive index of coverslips seems to vary (Tab. 4; Setterington 1953, tab. 1; Spinell & Loveland 1960, tab. 3; Norris 1961; Loveland & Centifanto 1986, p. 225). Coverslips come in different ranges of thickness indicated by a number, usually 0, 1, 1.5, 2, and 3, other thicknesses are nowadays available from a manufacturer on request (Tab. 3; Adam & Czihak 1964, p. 158; Sanderson 1994, p. 197). The coverslip thickness of a given number varies with the manufacturer and even within a batch (Tab. 3; Wagstaffe & Fidler 1955, p. 195; Spinell & Loveland 1960; Norris 1961; Thornton *et al.* 1985). Older coverslips may also vary in thickness within one and the same specimen (Spinell & Loveland 1960; Norris 1961). For these reasons and because several manufacturers around 1952 indicated the thickness of their coverslips would be 0.18 mm, the Standards Committee of the Royal Microscopical Society specified the desired thickness of coverslips to 0.18 mm in 1953 (Setterington 1953). Thornton *et al.* (1985) claimed that in the 1980s American, British, and Japanese microscope manufacturers corrected their objectives for a thickness of 0.18 mm (see also White 1974; Gill & Frost 1982), but this cannot be confirmed for microscopes from Nikon and Olympus for the 1980s and later based on images of such microscopes seen in the internet and comments received from Olympus Deutschland GmbH and Nikon GmbH (Kunerth pers. com.; Wilke pers. com.; Neuhaus pers. obs.). Continental microscope manufacturers like Leica/Leitz, Wild, and Zeiss correct at least their post-World War II objectives consistently for 0.17 mm thickness (Gill & Frost 1982).

Several aspects influence the quality of the microscopic image. Objectives are generally optimized concerning their resolution and contrast for a coverslip of 0.17 mm thickness and for the plane of focus immediately below the coverslip (Gibson & Lanni 1991; Lacey 1999; Keller 2006), except if the objective can be used with or without a coverslip. However, older and recent (plan)achromatic objectives with a nA < 0.5 as well as older Neofluar objectives with a nA < 0.5, e.g., objectives designed by Zeiss for a tube length of 160 mm, can usually be used with or without a coverslip, whereas most higher corrected objectives like Plan-Neofluar and Plan-Apochromat for biological studies with transmitted light and microscopes showing an infinite tube length require now a coverslip (e.g., see Zeiss Online Shop > Objective Assistant: Available from: https://www.micro-shop.zeiss.com). With increasing distance from the coverslip and with a biological specimen and a mounting medium both possessing a different refractive index than the immersion oil, contrast and resolution decrease significantly; spherical aberration is obviously already different at a focal depth of 4 µm in comparison to 0 µm using a planapochromat 63x/1.4 oil immersion objective (Keller 2006, p. 147, fig. 7.6). The situation may become worse if the wrong coverslip thickness or the wrong type of immersion oil is used or if the layer of the mounting medium is too thick (North 2006, p. 13). In addition, because of the different refractive indices of different cells and their components, the plane of focus does not really represent a "plane" (Pawley 2006). Also, a total mount of a specimen in a permanent mounting medium commonly bulges the coverslip slightly upwards (Woelke & Göke 1984, p. 212; Hooper 1986b, p. 318) and consequently changes the plane of focus slightly; this can be observed if the slide is held obliquely with light radiating from a side. A coverslip may be wedged revealing a thickness difference of up to 80 µm across 22 mm (Spinell & Loveland 1960, p. 61; Norris 1961). In accordance with theoretical and experimental considerations (Keller 2006; North 2006), a total mount of a (meiofaunal) specimen on a standard

glass slide cannot be observed in all necessary detail on the reverse side opposite to the coverslip, knowing that individual kinorhynch specimens mounted on microscope slides may range from less than 25–110 µm in thickness (Neuhaus pers. obs. on species of *Wollunquaderes* and *Pycnophyes*; Gill 2013, p. 283; Neuhaus 2013, p. 308). The limitations of standard glass slides can be partly overcome by inverting the glass slide and using long-distance objectives (for drawbacks see chapter 3.12 Study of specimens).

Coverslips of the selected thickness of 0.17 mm (No. 1.5H: Tab. 3) are available but more expensive. They are usually recommended for confocal laser scanning microscope studies, also because specimens are often mounted in liquid media leaving little space between the specimen and coverslip and because depth-dependent changes in spherical aberration can be corrected with deconvolution software for a given objective with known optical properties (Keller 2006; North 2006). However, such software correction is not available for most objectives using white transmitted light in bright field, phase contrast, or differential interference contrast microscopy. Actually, the thickness 0.17 mm engraved on an objective refers to the thickness of the coverslip plus the thickness of the mountant between the coverslip and specimen (see also White 1974, p. 411; Gill & Frost 1982; Gill 2013, p. 280). This layer of mounting medium may comprise a thickness of 5–76 µm measured in various slides with histological sections (Aumonier & Setterington 1967; White 1974, p. 417). Consequently, the coverslip should be slightly thinner (!) than 0.17 mm (Gill & Frost 1982), especially if information has to be obtained from deeper below the surface of the specimen.

In agreement with Gill & Frost (1982), the senior author therefore recommends to choose No. 1 (nowadays usually 0.13–0.16 mm) coverslips, which allows less blurred observations at focal planes deeper below the coverslip. A No. 0 (0.085–0.13 mm) coverslip has been suggested repeatedly (Krantz 1978; Hooper 1986a; Martin 1999; Walter & Krantz 2009), but these seem to be quite thin and would be beneficial only for rather thick preparations at the expense of a lower image quality of the specimen area closer to the coverslip. Coverslips thicker than 0.2 mm do not allow high aperture, high magnification oil immersion objectives to focus properly on the specimen because of their low working distance (Adam & Czihak 1964, p. 158). In any case, a compromise between optimal optical quality in a focal plane just below the coverslip and in a focal plane deeper in the specimen has to be made by choosing a certain coverslip thickness. In older museum collections, coverslips of a broad range of thicknesses can be expected, certainly much broader than mentioned in Table 3. Circular coverslips should be preferred over square ones for mounts of small entire specimens, because sealing the mount on a slide-ringing turntable with a fine brush is considerably easier and quicker (Wagstaffe & Fidler 1955, p. 193; Travis 1968; Hooper 1970; Gerakaris 1984; Sanderson 1994, pp. 203–204; Brown 1997; Hooper *et al.* 2005; Orajay 2005a, 2005b). Turntables seem to have been invented about 1850 by Shadbolt (Bracegirdle 1978, p. 115). Abu-Gharbieh & Smart (1969) suggested using a small polyethylene bottle instead of a brush for applying Glyceel, a technique revived by Wu (1986). The bottle should be stored top-down in order to prevent clogging of the bottle's orifice.

In the Museum für Naturkunde Berlin, a few microscope slides do not reveal a coverslip, because the slides show unfinished, not de-paraffinized, and unstained histological sections (Fig. 5F) or the scientist did not care (Fig. 5D). Occasionally, a specimen is mounted in air, and the coverslip fastened to the slide with strips of transparent tape and a small amount of mounting medium (Fig. 5E). In one case, a specimen was found to be mounted between two glass slides (Fig. 3J).

Cheap synthetic resin coverslip alternatives to glass coverslips have been applied since the 1920s, e.g., cellophane (a nitrocellulose derivate), Cerric lacquer D.010, Diatex (poly(methyl methacrylate)), gelatin plates, poly(*iso*-butyl methacrylate), Polyflex (a polystyrene), "slide strip plastic spray", X-ray radiation plates, and various lacquers (Kernohan 1928; O'Brien & Hance 1940; Perry 1954; Spinell & Loveland 1960; Streble 1963; Zimmerman 1963; Adam & Czihak 1964, pp. 159–161). The synthetic coverslip is applied either as a strip, by pouring or dropping the uncured liquid on the slide, or by dipping the slide in a solution of the uncured polymer. Coverslips made of polyolefin plastic have been developed for cell biology allowing cells to grow on them. These Thermanox™ slides come in different sizes and are 0.2 mm thick (e.g., https://tedpella.com). Synthetic coverslips are not recommended for permanent mounts, because the coverslips are quite thick (Thermanox™), of variable thickness, vulnerable to mechanical stress resulting in scratches, may interfere with observation under differential interference contrast, possess different optical qualities compared to modern borosilicate glass coverslips (Gill & Frost 1982), and will deteriorate considerably quicker than any glass coverslip. Diatex is dissolved by various immersion oils over time (Streble 1963), but this may be true for other synthetic resins as well.

TABLE 3. Thickness of coverslips according to various sources.

Coverslip No.	Coverslip thickness [mm]	Remarks	Source
1	0.1016	Chance Brothers and Co., Ltd., March 1859	Anonymous 1941
2	0.1587		
3	0.254		
extra thin	0.075–0.100	Chance Brothers and Co., Ltd., 1941	
1	0.100–0.167		
2	0.167–0.215		
0	0.07–0.13	industrial standard	Wagstaffe & Fidler 1955, p. 195
1	0.13–0.17		
2	0.17–0.21		
3	0.21–0.35		
1.5	0.152–0.192	Chance Brothers and Co., Ltd., measured	Spinell & Loveland 1960
1.5	0.162–0.217	manufacturer Corning, measured	
0	0.075–0.10	British classes	Adam & Czihak 1964
1	0.10–0.167		
2	0.167–0.215		
3	> 0.215		
0/1	0.08–0.12	German classes: manufacturer O. Kindler	
1	0.12–0.17		
2	0.17–0.25		
0	0.085–0.130		White 1974; Sanderson 1994
1	0.130–0.160		
1.5	0.160–0.190		
2	0.190–0.250		
3	0.250–0.350		
0	0.085–0.130	industrial standard	Thornton *et al.* 1985
1	0.129–0.170		
1.5	0.160–0.191		
2	0.191–0.231		
3	0.254–0.320		
0	0.088–0.126	measured	
1	0.120–0.195		
1.5	0.152–0.212		
2	0.194–0.235		
0	0.085–0.115	manufacturer Menzel	Available from: http://www.menzel.de/files/coverglass-d-263-m-en.pdf (accessed 19 January 2016)
1	0.130–0.160		
1.5	0.160–0.190		
2	0.195–0.225		
1.5H	0.165–0.175	manufacturer Zeiss, high performance coverslips	Available from: https://www.micro-shop.zeiss.com (accessed 18 March 2016)

TABLE 4. Refractive indices of glass, immersion oil, water, animal tissues, selected mounting media (measurements of dry solidified medium except glycerol), and selected physical clearing agents. The temperature is given in [ ] if available.

Object	Refractive index nD	Remarks	Source
coverslip	1.531	Corning	Loveland & Centifanto 1986, p. 225
	1.523–1.533		Norris 1961
	1.5131–1.5318 [20/25°C]	coverslips from the years 1942–1959	Setterington 1953, tab. 1; Spinell & Loveland 1960, tab. 3
borosilicate coverslip	1.5230		Menzel, product information. Available from: http://www.menzel.de/files/coverglass-d-263-m-en.pdf (accessed 19 January 2016)
anise oil (= anisole)	1.516 [20°C]	previously used as immersion oil	Available from: http://www.chemicalbook.com/ChemicalProductProperty_EN_CB5100716.htm (accessed 06 April 2016)
Leica Immersion Oil, No. 11 513 859	1.518 [23°C]		label on original bottle
Zeiss Immersol™ 518 F, Zeiss Immersol™ 518 N	1.518 [23°C]		Available from: https://www.micro-shop.zeiss.com (accessed 30 March 2016)
water	1.333 [20°C]		White 1970, p. 257
Animal tissues			
live cultivated smooth muscle cells	1.356–1.364 [23°C]		Curl *et al.* 2005
living cell	1.35–1.36		Crossmon 1949; Berland 1984
fixed cell	1.50	refractive index depends on fixative	Crossmon 1949; Berland 1984
fixed unstained histological tissue	1.536		Crossmon 1949, p. 243
	1.534–1.540		Groat 1940
	1.530–1.540		Lillie *et al.* 1953, p. 57
	1.55		Marshall 1932
	1.53–1.56		Krauter & Rüdt 1980
frozen tissue	1.380–1.467		Dirckx *et al.* 2005
arthropod endocuticle	1.50–1.53		Singer 1967
arthropod exocuticle	1.53–1.56		Singer 1967
arthropod epicuticle	1.56–1.59		Singer 1967

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TABLE 4. (Continued)

Object	Refractive index nD	Remarks	Source
Mounting media			
Allen's medium	1.425	gum-chloral medium	Gray & Wess 1950, p. 288
André and Langeron's medium	1.472	gum-chloral medium	Gray & Wess 1950, p. 288
Apathy's medium	1.450	gum arabic medium	Gray & Wess 1950, p. 288
Aquatex®	1.390–1.400 [20°C]		Merck[1]
Aroclor® 1242	1.627–1.629		Loveland & Centifanto 1986, p. 231
Aroclor® 1254	1.639–1.641		McCrone 1984
Aroclor® 1260	1.651 [20°C]		McCrone 1984
	1.647–1.649 [20°C]		Loveland & Centifanto 1986, p. 231
Aroclor® 1262	1.6501–1.6517 [20°C]		Loveland & Centifanto 1986, p. 231
Aroclor® 4465	1.664–1.667 [20°C]		Loveland & Centifanto 1986, p. 231
Aroclor® 5442	1.662 [20°C]		McCrone 1984
Aroclor® 5460	1.673 [20°C]		McCrone 1984
	1.660–1.665 [20°C]		Loveland & Centifanto 1986, p. 231
Berlese's medium	1.485	gum-chloral medium	Gray & Wess 1950, p. 288
Bio-Plastic®, Polylite®	1.555–1.562	depending on amount of catalyst	Senior 1970
Caedax	1.558 [20°C]	see text	Deutsch 1962
	1.5730 [22°C]	see text	Loveland & Centifanto 1986, pp. 187, 231
Caedax A	1.6724 [20°C]	calculated: 1.6673	Lillie *et al.* 1953, p. 69, tab. 1A
Caedax 547	1.63		Engbert 1957
Canada balsam	1.535 [25°C]		Groat 1939
	n. a.	1.5322 with xylene	Greco 1950
	1.5447 [20°C], calculated	with xylene: 1.5232 [20°C]	Lillie *et al.* 1953, tab. 1A
	1.5411.547 [20°C]		Adam & Czihak 1964
	1.5181.521 [20°C]		Bender 1967
	1.532	with xylene: 1.497	Brown 1997

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TABLE 4. (Continued)

Object	Refractive index nD	Remarks	Source
cellulose caprate	1.4734		Lillie & Greco Henson 1955
Chevalier's medium	1.388	gum-chloral medium	Gray & Wess 1950, p. 288
Clarite X (= Nevillite I)	1.5647 [20°C]	not available after 1950	Lillie *et al.* 1953, tab. 1A
C-M Medium	1.428	dry medium?	Clark & Morishita 1950
Coumarone-indene resin	1.6209 [20°C], calculated		Lillie *et al.* 1953, p. 67
gum dammar	1.559	= gum damar	Loveland & Centifanto 1986, p. 187
Davies's medium	1.433	gum-chloral medium	
Diaphane	1.5486 [20°C]		Lillie *et al.* 1953, p. 61
Dimethyl hydantoin formaldehyde (DMHF)	1.54		Steedman 1958; Bameul 1990, p. 235
	1.52–1.54		Steedman 1976c, p. 190
Doetschman's medium	1.415	gum-chloral medium	
Entellan® new	1.490–1.500 [20°C]		EMS[2]
Eukitt™	1.491–1.497		Ant 1959
	1.510		EMS[2]
	1.515		Richardson 2014, p. 17
Eukitt™ Neo Special Mounting Medium	1.48 [20°C]		EMS[2]
Euparal	1.5174 [20°C]	with Euparal Essence: 1.4776 [20°C]	Lillie *et al.* 1953, p. 64
	1.535		Böck 1989, p. 297
Faure's medium	1.437	gum-chloral medium	Gray & Wess 1950, p. 288
Gater's medium	1.485	gum-chloral medium	Gray & Wess 1950, p. 288
glycerol	1.474 [20°C]		Adam & Czihak 1964, p. 170
glycerol-gelatin (Kaiser's)	1.4353 [20°C]	data for dry medium?	Greco 1950
Highman's medium	1.417	gum-chloral medium	Gray & Wess 1950, p. 288

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TABLE 4. (Continued)

Object	Refractive index nD	Remarks	Source
Hogg's medium	1.380	gum-chloral medium	Gray & Wess 1950, p. 288
Hoyer's medium	1.419	gum-chloral medium	Gray & Wess 1950, p. 288
Hyrax	1.71 [20°C]		Loveland & Centifanto 1986, p. 232
Langerhans's medium	1.462	gum-chloral medium	Gray & Wess 1950, p. 288
Lillie & Ashburn's medium	1.385	gum-chloral medium	Gray & Wess 1950, p. 288
Martin's medium	1.428	gum-chloral medium	Gray & Wess 1950, p. 288
Morrison's medium	1.440	gum-chloral medium	Gray & Wess 1950, p. 288
Naphrax™	1.76–1.8		Fleming 1943
	1.710	toluene-free: 1.67	Thorns[3]
	1.66		Rosenfeldt[4]
Novolacs	1.65 [20°C]	BRPB 5215	Crumpton & Wetzel 1980, 1981
Permount™ (old)	1.5376 [20°C]	formula before 1953	Greco 1950m p. 12; Lillie *et al.* 1953, tab. 1A
poly(vinyl acetate)	1.466		Loveland & Centifanto 1986, p. 193
polyvinyl chloralphenol	1.496 [22°C]	data for dry medium?	Ribeiro 1962
polyvinyl chloral hydrate-lactophenol	1.414 [20°C]	data for dry medium?	Heinze 1952
Rhenohistol	estimated: 1.530 [20°C], calculated: 1.533 [20°C]	calculation by Groat in Lillie *et al.*, 1953	Lillie *et al.* 1953, p. 70
Robin: Medium A	1.402	gum-chloral medium	Gray & Wess 1950, p. 288
Robin: Medium B	1.408	gum-chloral medium	Gray & Wess 1950, p. 288
Robin: Medium C	1.390	gum-chloral medium	Gray & Wess 1950m p. 288
Swan's medium	1.470	gum-chloral medium	Gray & Wess 1950, p. 288
Technovit® 7100	1.505		Kulzer[5]
Visikol™	1.4450		Villani *et al.* 2013

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TABLE 4. (Continued)

Object	Refractive index nD	Remarks	Source
Physical clearing agents			
anise oil	1.556 [20°C]	= anisole; see also under immersion oil above	White 1970
bergamot oil	1.463 [20°C]		White 1970
cajeput oil	1.468 [20°C]		White 1970
cedar wood oil	1.511 [20°C]		White 1970
cinnamon oil	1.584 [20°C]		White 1970
clove oil	1.532 [20°C]		White 1970
creosote	1.538 [17°C]		Behrens 1892, p. 42
limonene	1.475 [20°C]		White 1970
methyl benzoate	1.51 [20°C]	= Niobe oil	Kiernan 1999, p. 42, 2015, p. 55
methyl salicylate	1.537 [20°C]	= wintergreen oil	White 1970
phenol	1.549 [17°C]		Behrens 1892, p. 42
terpineol	1.48 [20°C]	= synthetic oil of lilac	Kiernan 1999, p. 42, 2015, p. 55
tert-butyl alcohol	1.39 [20°C]		Kiernan 1999, p. 42, 2015, p. 55
Zeiss L15	1.515		Zölffel pers. com.
Zeiss L25	1.525		Zölffel pers. com.
Zeiss W15	1.515		Hirsch & Hager 1955, p. 152; Zölffel pers. com.

[1] Merck: Available from: http://www.merckmillipore.com/DE/de/product/Aquatex%C2%AE,MDA_CHEM-108562#anchor_BRO (accessed 21May 2015).

[2] EMS: = Electron Microscopy Sciences. Available from: http://www.emsdiasum.com/microscopy/products/histology/mounting_media.aspx (accessed 21May 2015 and 04 April 2017).

[3] Biologie-Bedarf Thorns: Available from: http://www.biologie-bedarf.de/products/382361/, http://www.biologie-bedarf.de/products/382371/ (accessed 09 July 2015).

[4] Dr G. Rosenfeldt: Available from: http://www.mikrohamburg.de/Tips/T_Hochbrechende%20Einschlussmittel.html (accessed 14 July 2015).

[5] Kulzer: Available from: http://kulzer-technik.de/en_kt/kt/maerkte/histologie/produktbereiche_1/polymerisationssysteme_1/technovit_7100.aspx (accessed 29 March 2016).

3.7 Mounting media

3.7.1 General aspects of mounting media

Different scientific users express different requirements for a mounting medium: (a) Many taxonomists need to mount entire specimens and often want the inner organs to be macerated in order to see cuticular structures more clearly, particularly in arthropods. Also, the refractive index should differ from that of glass and from that of the specimen. Upton (1993) revealed the noteworthy history of some of the macerating media, the gum-chloral media, their formulae, applications, and problems. (b) Especially diatom specialists need a much higher refractive index than the regular media offer. (c) Histologists require information about compatibility of stains with the mounting medium and a refractive index close to that of the tissue (Lillie *et al.* 1950, 1953; Gill 2013, p. 262; Ravikumar *et al.* 2014). Lillie *et al.* (1950, 1953) investigate the suitability of several mounting media for stained histological sections. (d) From a curatorial point of view, the following characters should be regarded as essential for a good mounting medium of entire biological specimens (see also Brown 1997; Ravikumar *et al.* 2014):

(1) The medium should reveal a refractive index allowing observation of the finest details of specimens.
(2) A solidifying medium should dry in a way that no cavities develop near the specimen and in the periphery of the coverslip originating from the loss of volume because of the evaporation of a solvent.
(3) The medium must not react with either the specimen, a potential stain, or the coverslip seal.
(4) The medium must remain stable over decades and especially not segregate into its components (= syneresis) or develop cracks, cavities, precipitations, crystals, and a granular background. The coverslip must not detach.
(5) The specimen should be re-mountable in case the slide mount is damaged or problems with the mounting medium occur.

The latter two criteria agree with similar general considerations in conservation science (e.g., Davison 2003, p. 208; Horie 2011, pp. 3–13). Horie (2011, p. 41) also states that "all polymers must be expected to oxidize, either quickly or slowly, over time" and suggests *inter alia* not to use polymers that contain plasticizers (e.g., nail varnish, Araldite) and shrink considerably on drying (e.g., gelatin; Horie 2011, p. 105–106). Conservation issues have rarely been considered in previous suggestions for ideal mounting media (Scott 1951; Lillie *et al.* 1953; Loveland & Centofanto 1986; Hrauda 1990; Koomen & Vaupel Klein 1995; Brown 1997; Ravikumar *et al.* 2014).

Mounting media may be grouped into fluid media (e.g., formaldehyde, glycerol, lactophenol) and solidifying media. The latter are water-soluble (e.g., Fluoromount G™, glycerol-gelatin, gum-chloral media, poly(vinyl alcohol), polyvinyl lactophenol), limited water-tolerant (e.g., Euparal), or hydrocarbon-soluble (e.g., neutral Canada balsam, Caedax, DPX/DePeX, Entellan®, Eukitt™) (Tab. 5). Generally, "highly volatile solvents are to be avoided if the development of air bubbles is to be prevented" in hydrocarbon-soluble mounting media (Lillie *et al.* 1950, p. 3). Therefore, xylene and toluene are preferred as solvents over benzene and chloroform (for vapor pressure see Tab. 10). Gas bubbles may also develop by drying mounts in an oven as has been reported for Clarite and a more recent formula of DPX (Mohr & Wehrle 1940; Göke 2000; Sluys pers. com.). Mounting media based on natural resins or ingredients like Canada balsam, Euparal, glycerol-gelatin, Malinol, Styrax, and Venetian turpentine as well as Hyrax and some media made of synthetic polymers such as Aroclor®, Caedax, Naphrax™, and Rhenohistol usually reveal a strong autofluorescence, whereas some synthetic resins do not like DePeX, DPX, Eukitt™, Entellan® new, and Fluoromount G™ as well as glycerol (Spurr 1954, p. 310; Deutsch 1962; Hrauda 1990; Roe *et al.* 1991; Göke 2000; Wiggins & Drummond 2007, p. 4). However, fluorescence from aniline blue-stained plant material does not interfere with the autofluorescence of Euparal (Ramanna 1973). A range of media such as Canada balsam, Caedax (not available anymore), DPX/DePeX, Entellan®, Eukitt™, Euparal, Fluoromount G™, and Permount™ can nowadays only be purchased from a manufacturer, but originally recipes for most of the media were published by scientists (Tab. 5). Few media may be simply mixed in a lab like all gum-chloral media, Kaiser's glycerol-gelatin, and polyvinyl lactophenol, whereas some media require extensive lab work and chemical reaction such as Coumarone, Hyrax, Naphrax™, and Pleurax (Tab. 5). Recipes of different mounting media can be found in Romeis (1948), Gatenby & Beams (1950), Gray (1954), Adam & Czihak (1964), Upton (1993), and Brown (1997). Numerous practical hints how to narcotize, fix, and stain specimens and to mount and seal a

microscope slide are provided by Gatenby & Beams (1950) and Gray (1954). The latter, Wagstaffe & Fiddler (1955), Adam & Czihak (1964), and Knudsen (1966) also described many taxon-specific techniques. For planktonic organisms, such information is found in Steedman (1976c).

It turns out that stained sections fade in unsuitable media even if stored in the dark for two weeks (Dewse & Potter 1975) or one to two months (Gill 2013, pp. 265–267; Schmolke 1993). Stains like Feulgen stain, methylene blue-azure II-basic fuchsin fade significantly by 20–44% in various mounting media within two weeks and five months, respectively, even if slides are stored in the dark, so observation within two weeks is strongly recommended (Dewse & Potter 1975; Humphrey & Pittman 1977). Similar results have been found for toluidine blue and pyronin G of Araldite sections mounted in several media within one month (Schmolke 1993). The latter author recommends not mounting sections at all in order to allow re-staining after an extended period of time. Whereas Feulgen stain fades most in Euparal (30%) and less so in Canada balsam (ca. 13%) and DePeX (ca. 17%), methylene blue-azure II-basic fuchsin fades most in Canada balsam (by 36–44%), but also in Permount™ (22–29%) and DePeX (26–39%). Fading may also appear primarily in the periphery of the coverslip (Fig. 4J), probably because of the impact of oxygen, gases, and vapors.

Because of problems of Canada balsam with stains on sections (see below), considerable efforts have been taken to develop new mounting media with the advent of new plastic polymers but also to test resins from other plants than the Canada fir, which delivers Canada balsam (see reviews by Southgate 1923; Wicks *et al.* 1946; Lillie *et al.* 1950, 1953). Among these plant resins tested for their suitability for mounting biological specimens on slides is Yucatan elemi (Southgate 1923). Many of the mounting media tested at that time did not perform much better than Canada balsam (Richards & Smith 1938; Groat 1940; Lillie *et al.* 1950, 1953; Gray & Wess 1951; Wirth & Marston 1968) and consequently disappeared from the market again. Application of Canada balsam requires time-consuming steps of dehydration and harmful chemicals like xylene, so notable energy was devoted to replace this medium by media requiring less time for preparing microscope slides and containing less noxious components (Salmon 1947, 1951a; Spurr 1954; Clifford & Lewers 1960; Bameul 1990). Virtually every plastic seems to have been tested, also, because new synthetic resins simply became available at a certain time or had not been tested before (Tabs 1, 5), e.g.,

- acrylic varnish (Lödl 1999),
- alkyd varnish (Hrauda 1990),
- aniline-formaldehyde-sulfur polymer (Hanna 1927, 1930),
- cyanoacrylates (Geysen & Loof 1983; Liu *et al.* 2010; Criado-Fornelio *et al.* 2014),
- dimethyl hydantoin formaldehyde resin (Steedman 1958, 1976c; Smith 1966; Bameul 1990),
- hydroxypropyl methacrylate (Leduc & Holt 1965; Crumpton 1987),
- isobutyl methacrylate (Groat 1940),
- methyl cellulose and poly(ethylene glycol) (Clark & Morishita 1950),
- methyl methacrylate (Richards & Smith 1938),
- naphthalene polymer like Coumarone and Naphrax™ (Fleming 1943, 1954; Frison 1952a, 1952b, 1955),
- phenol-formaldehyde resin like Novolac (Crumpton & Wetzel 1980, 1981),
- phenol-sulfur-sodium sulphide polymer like Pleurax (Hanna 1949; Hepworth 1994),
- polychlorinated biphenyls and polyphenyls in variable composition like Aroclors® (Frison 1955; Hasle & Fryxell 1970; Göke 1973, 2000; McCrone 1984),
- polystyrene (Kirkpatrick & Lendrum 1939, 1941),
- polyester (Senior 1970),
- polyterpene like Piccolyte® (Wicks *et al.* 1946),
- polyurethane (Denton 1987),
- poly(vinyl acetate-co-vinyl chloride) (Skiles & Georgi 1937; Gray & Wess 1951; Lillie *et al.* 1953),
- poly(vinyl alcohol) (Downs 1943; Salmon 1947, 1951a, 1951b, 1954),
- poly(vinyl pyrrolidone) (Steedman 1976c; Larsen 1980; Villani *et al.* 2013; Simon *et al.* 2015),
- trimethoxysilane (Criado-Fornelio *et al.* 2014), and
- urea-formaldehyde resin (Kaudewitz 1951–1952; Krauter 1952–1953; Ant 1957; Ossiannilsson 1958).

TABLE 5. (Potential) contents of selected mounting media. Long-time stable or reversible media indicated by an asterisk and printed in bold.

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
Aquatex®	water	poly(1-vinyl-2-pyrrolidone)	glycerol?				Fig. 12; Merck[1], VWR[2]; Steedman 1976c, p. 191
Araldite CY212		4,4'-isopropylidene diphenol (a diglycidyl ether of bisphenol A), hardener: dodecenyl succinic anhydride	dibutyl phthalate			accelerator: benzyl dimethyl amine; flexibilizer: 2,2'-[oxybis[(methyl-2,1-ethanediyl) oxymethylene]]bis-oxirane (= DER736)	SPI.Supplies[20]; Sanderson 1994, pp. 65–66
Bio-Plastic®, Polylite®	32.2% styrene	62% polyester pre-polymer, < 5.5% methacrylate monomers, styrene				catalyst: methyl ethyl ketone peroxide	CAMEO[13], Aldon[14]; Senior 1970
Caedax, Caedax A, Caedax 547	xylene	cyclohexanone formaldehyde (here: Resin AW2)	polychlorinated biphenyl (here: Clophen A60®)			see text	Kern *et al.* 1946; Lillie *et al.* 1953, p. 69, tab. 1A; Engbert 1957; Deutsch 1962; Krauter & Rüdt 1980; Loveland & Centifanto 1986, pp. 187–188, 231
* **Canada balsam**	xylene; partly volatile components of balsam (Δ^3-carene, levopimaric acid, limonene, myrcene, palustric acid, β-phellandrene, α-pinene, β-pinene)	balsam (abienol, abietic acid, isopimaric acid, sandaracopimaric acid)			dehydroabietic acid, isopimaric acid, neoabietic acid, sandaracopimaric acid	neutralization: potassium carbonate; resin from *Abies balsamea* (Linné, 1758)	Fig. 6; Lombard *et al.* 1958; Gray & Mills 1964; Bender 1967; Böck 1989, p. 296; Mills & White 1999, p. 101
cellulose caprate	xylene	cellulose caprate (= cellulose tri-decanoate)	poly(α-methyl styrene), *n*-cyclohexyl *p*-toluenesulfonamide			developed as optical cement	Lillie & Greco Henson 1955; Field 1958; Loveland & Centifanto 1986, p. 194

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TABLE 5. (Continued.)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Maceratin g agent	Preservative	Remarks	References
Clarite (= Nevillite V), Clarite X (= Nevillite I)	xylene; Groat 1939, Gatenby & Beams 1950, p. 216: toluene	cycloparaffin polymer	Mohr & Wehrle 1942: dibutyl phthalate			not available after 1950	Groat 1939; Mohr & Wehrle 1940, 1942; Lillie *et al.* 1950, 1953
C-M Medium	ethanol, water	methocellulose (= methyl cellulose)	poly(ethylene glycol) (here: carbowax 4000), diethylene glycol	lactic acid		see text	Figs 12, 13; Clark & Morishita 1950; Horie 2011, p. 192
CMC-9 /CMC -10 (= carboxy methyl cellulose?)	water	fully hydrolyzed poly(vinyl alcohol)		lactic acid		CMC(P)-9: low viscosity, CMC(P)-10: high viscosity; CMC(P)-9AF or -10AF: pretinted with acid fuchsin; CMC-S (= stain); see text	Figs 12, 13; EMS[3]; Polysciences[4]; Mikkelsen 1985
CMCP-9 /CMCP -10 (= carboxy methyl cellulose phenol?)	water (CMCP-9: 51–60%, CMCP-10: 71–80%)	fully hydrolyzed poly(vinyl alcohol) (CMCP-9: 0–5%; CMCP-10: 6–10%)		21–30% lactic acid	21–30% phenol		Figs 12, 13; EMS[3]; Polysciences[4]; Becker & Heard 1965; Knudsen 1966, p. 501; Beckett & Lewis 1982
DePeX	25–50% xylene	distrene 80 (a polystyrene)	2.5–10% dibutyl phthalate			DePeX and DPX seem to differ only in the "relative proportions of polystyrene and dibutyl phthalate" (Roe *et al.* 1991)	Figs 12–14; EMS[3]; Kirkpatrick & Lendrum 1939, 1941; Roe *et al.* 1991; Kiernan, 1999, 2015; Ravikumar *et al.* 2014
DPX (= Distrene Plasticizer Xylene)	xylene	distrene 80 (a polystyrene)	dibutyl phthalate, tri-*o*-cresyl phosphate (older formula)				
dimethyl hydantoin formaldehyde (DMHF)	ethanol, water	dimethyl hydantoin formaldehyde resin			(phenol if medium lacks ethanol to prevent growth of fungi)	commercial version: Gurr's Michrome Aquamount	Steedman 1958, 1976c, pp. 189–190; Bameul 1990; Richardson 2014, p. 18
Entellan®	50–75% xylene	25–50% poly(*n*-butyl methacrylate)					Fig. 12; EMS[3]
Entellan® new	50–70% xylene	poly(methacrylate-co-butyl methacrylate)					Fig. 12; VWR[2]
Eukitt™	55% xylene	45% poly(*n*-butyl methacrylate-co-methyl methacrylate)				identical with Corbit balsam	Fig. 12; EMS[3]; Sigma-Aldrich[5]; Schmolke 1993

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TABLE 5. (Continued)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
Eukitt™ Neo Special Mounting Medium	(R)-*p*-mentha-1,8-diene	poly(*n*-butyl methacrylate-co-methyl methacrylate)					Fig. 12; EMS[3]
*** Euparal (2 versions: transparent and green = vert)**	eucalyptol, paraldehyde; partly volatile components of gum sandarac (limonene, α-pinene, β-pinene)	compounds of gum sandarac (communic acid, manool, polycommunic acid, sandaracopimaric acid, 12-acetoxy-sandaracopimaric acid, sugiol, torulosic acid, torulosol, totarol)	camsal (= camphor, phenyl salicylate = salol)		paraldehyde	clearing agent: methyl salicylate; color in Euparal green: copper salt (copper abietinate?); sandarac resin from *Tetraclinis articulata* (Vahl, 1791)	Figs 7, 8; Gilson 1906; Shepherd 1918; Lillie *et al.* 1953, p. 64; Wagstaffe & Fidler 1955, p. 175; Gough 1964; Böck 1989, p. 297; Mills & White 1999, p. 99; Scalarone *et al.* 2003a
Fluoromount G™	water	10% poly(vinyl alcohol)	water bound to glycerol		0.1% sodium azide	buffer: disodium hydrogen phosphate (Na_2HPO_4); clearing agent: 25–50% glycerol	Fig. 12; EMS[3]; Spurr 1954; Witte 1976
***glycerol mount**	glycerol	-	-	-	-	clearing agent: glycerol; seal: paraffin	Fig. 13; Adam & Czihak 1964, pp. 162, 170
glycerol-gelatin (Kaiser's)	water	gelatin	glycerol			clearing agents: phenol, glycerol	Merck[1]; Adam & Czihak 1964, p. 162; Greco 1950; Steedman 1976c, pp. 190–191; Hevers 1985
glycerol-gelatin (Zirkle's aceto-carmin)	water	gelatin, dextrose	glycerol	glacial acetic acid (also fixative)		stain: carmine; clearing agent: glycerol; iron trichloride ($FeCl_3$)	Zirkle 1937, 1940
gum-chloral media like Hoyer's mounting medium	glycerol, water	compounds of gum arabic (polysaccharide with arabinose, galactose, rhamnose, and glucuronic acid as calcium, magnesium, and potassium salt; 2 glycoproteins; galacturonic acid)		chloral hydrate		Higgins 1986, Amrine & Manson 1996: contrast enhancer iodine and potassium iodide; gum arabic from *Acacia senegal* (Linné, 1758)	Fig. 13; Higgins 1971, 1977, 1982, 1983, 1986; Upton 1993; Amrine & Manson 1996; Mills & White 1999, p. 77; Dror *et al.* 2006; Faraji & Bakker 2008

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TABLE 5. (Continued)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
modified gum-chloral medium with BTDA	glycerol, water	benzophenone-3,3',4,4'-tetracarboxylic dianhydride (= BTDA)		acetic acid, chloral hydrate		sorbitol; Amrine & Manson 1996: contrast enhancers iodine and potassium iodide, chlorazol black E, lignin pink, toluidine blue	Fig. 14; Keifer 1979; Amrine & Manson 1996
lactophenol gel	water	methyl cellulose	ethylene glycol	lactic acid		clearing agent: phenol	Figs 12, 13; Zander 1983
Loctite UV 357		polyurethane				cured by UV-light; for bonding glass to glass; no further information Available from: manufacturer	Denton 1987
Loctite® 363™ Impruv® Potting Compound Light Cure	2004, 2007: 5–10% acrylic acid, 0.1–1% ethylene glycol; 2010: < 6% acrylic acid; 2014: 5–10% acrylic acid	2004, 2007: 30–60% high boiling methacrylate, 30–60% poly(urethane methacrylate), 1–5% hydroxyalkyl methacrylate, 1–5% γ-glycidoxypropyl trimethoxysilane 2010: 30–60% dicyclopentenyl oxyethyl methacrylate, 10–30% polyurethane methacrylate resin, 1–5% hydroxyalkyl methacrylate, 1–5% 1-hydroxycycohexyl phenyl ketone; 2014: 30–60% acrylate, 30–60% polyurethane methacrylate resin, 1–5% 2-hydroxyethyl methacrylate, substituted silane				photo initiator; medium cured by UV light	KITCOFO 14.XII.2004[15], EDHOY 21.V.2007[16], MYHENKEL 20.VII.2010[17], EDHOY 19.XI.2014[18]; Silverman 1986

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TABLE 5. (Continued)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
Malinol	40–60% xylene, 5–15% ethylbenzene	compounds of synthetic Canada balsam (possibly Zanzibar copal)					Nutriplate[7]; Krauter & Rüdt 1980
Naphrax™	toluene; alternatively in older formulae: benzene, xylene; Thorns[8]: toluene-free formula available	naphthalene-formaldehyde resin	dibutyl phthalate, tri-*o*-cresyl phosphate, or triphenyl phosphate	glacial acetic acid		catalyst : sulfuric acid; neutralization: sodium carbonate (Na_2CO_3); prepared by heating	Figs 13, 14; Thorns[8]; Fleming 1943, 1954
Naphrax™	toluene	naphthalene				paraformaldehyde, glacial acetic acid, *p*-toluenesulfonic acid, neutralization: calcium carbonate ($CaCO_3$); prepared by heating	Rosenfeldt[9]
Permount™ (new: formula after 1953)	limonene, α-pinene, β-pinene, β-phellandrene, toluene; Lillie *et al.* 1953: xylene	polyterpene resin based on limonene, α-pinene, β-pinene				anti-oxidant 2-6-di-*tert*-butyl-*p*-cresol for minimizing crazing and fading of stain; see text	Figs 6, 13; Bioworld[6], EMS[3]; Fisher[12]; Lillie *et al.* 1953, p. 66; Hollander & Frost 1971
Pertex	xylene	different poly(butyl acrylates)					TAAB[10]
Piccolyte®	xylene	hydrogenated polyterpene resin based on β-pinene					Wicks *et al.* 1946
poly(vinyl pyrrolidone) medium	water or phosphate buffer (sodium dihydrogen phosphate, disodium hydrogen phosphate) or TRIS buffer (tris(hydroxymethyl) aminomethane, HCl)	poly(vinyl pyrrolidone)	glycerol		thymol		Figs 12, 13; Kiernan 1999, p. 54, 2015, p. 69

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TABLE 5. (Continued)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
polyvinyl lactophenol	water; Heinze 1952: glycerol	poly(vinyl alcohol)	(water bound to glycerol)	lactic acid, phenol; Heinze 1952: chloral hydrate	phenol		Figs 12, 13; Downs 1943; Salmon 1947, 1951a, 1951b; Heinze 1952; Lipovsky 1953; Rutledge 1954; Spurr 1954; Witte 1976; Woelke & Goeke 1984
Polyviol	ethanol, water	mixture of 2 poly(vinyl alcohols) (here: Mowiol® N 4-98 and N 56-98)		lactic acid		older formula: Polyviol 17 (here: Mowiol® N 30-98 and N 90-98)	Figs 12, 13; Danielsson 1985
Rhenohistol	xylene	condensation product of aliphatic ketone and formaldehyde (here: Emekal 65)	plasticizer				Schiller 1949; Lillie *et al.* 1953
Styrax	acetophenone, benzaldehyde, 4-hydroxybenzenepropanol, benzoic acid, caryophyllene, hydrocinnamyl alcohol, *trans*-cinnamyl aldehyde, limonene, 1-phenyl-1-ethanol, 3-phenyl-2-propanol, α-pinene, β-pinene, styrene	camphene, cinnamic acid, *p*-hydroxycinnamic acid, *trans*-cinnamyl alcohol, oleanolic acid, oleanonic acid, phenylpropyl cinnamate, resorcinol, styracin				storax resin from *Liquidambar orientalis* Miller, 1768	Fig. 9; Beck 1959; Hafizoğlu 1982; Hafizoğlu *et al.* 1996; Modugno *et al.* 2006; Kim *et al.* 2008
Technovit® 7100 and hardener 1 or hardener 2		poly(2-hydroxyethyl methacrylate)	Gerrits & Smid 1983: poly (ethylene glycol) 400			hardener 1: dibenzoyl peroxide, dicyclohexyl phthalate; hardener 2: 2013: N,N,3,5-tetramethyl aniline 2014: 5-butylbarbituric acid, chloride ions; dimethyl sulfoxide in German MSDS only	Fig. 12; EMS 26.XII.2015[19]; Kulzer[11]; Gerrits & Smid 1983

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TABLE 5. (Continued)

(Trade) name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Macerating agent	Preservative	Remarks	References
Venetian turpentine medium (Wilson)	water, partly volatile components of Venetian turpentine (allocimene, ³-carene, caryophyllene, elemol, β-eudesmol, larixyl acetate, larixol, limonene, longifolene, levopimaric/ palustric acid, α-pinene, β-pinene, α-terpineol, verbenone)	compounds of Venetian turpentine (abietic acid, epimanool, isopimaric acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethenyl)-phenanthrenecarboxylic acid, 7-oxo-dehydroabietic acid, pimaric acid, sandaracopimaric acid)		acetic acid, propionic acid	phenol, dehydroabietic acid, isopimaric acid, neoabietic acid, sandaracopimaric acid	Venetian turpentine (= Venice turpentine) from *Larix decidua* Miller	Figs 10, 11, 13; Wilson 1945; Mills & White 1999, pp. 100–102, tab. 8.3; Scalarone *et al.* 2002
Visikol™	a C1–C6 alcohol, glycerol, trichloroethanol	poly(vinyl pyrrolidone)		trichloroacetic acid, one or more inorganic and organic acids	a preservative	additional compounds like a stabilizer	Figs 12, 13; Villani *et al.* 2013; Simon *et al.* 2015
Water-glass-Glycerol	water	water-glass (= sodium silicate)	glycerol			see text	Fig. 12; Creaser & Clench 1923; Zander 2014
Zeiss L15, L25, W15	organic solvent (L15, L25) or water (W15)	ester of phthalic anhydride and glycerol	glycerol?			L15 and L25 solidify, W15 remains liquid	Figs 13, 14; Hirsch & Hager 1955; Anonymous 1960

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[9] Dr G. Rosenfeldt: Available from: http://www.mikrohamburg.de/Tips/T_Hochbrechende%20Einschlussmittel.html (accessed 14 July 2015).
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[11] Kulzer: Available from: http://kulzer-technik.de/en_kt/kt/maerkte/histologie/produktbereiche_1/polymerisationssysteme_1/technovit_7100.aspx (accessed 29 March 2016), http://kulzer-technik.de/de_kt/kt/maerkte/metallographie/produktbereiche/verbrauchsmaterialien_einbetten_1/technovit__7100.aspx (accessed 30 October 2015).
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[20] SPI.Supplies: Available from: https://www.2spi.com/item/z02830/ (accessed 13 April 2016).

TABLE 6. Advantages and disadvantages of selected mounting media based on references about microscope slides and unpublished observations by various persons. Long-time stable or reversible media are marked by an asterisk and printed in bold.

(Trade) name	Advantages	Disadvantages	References
Aquatex®	- specimen can be mounted directly from water	- formation of cavities and cracks (Fig. 15C)	Merck[2]; this paper
Bio-Plastic®, Polylite®	- medium lasts > 1 year, - robust slide, - fast green F.C.F. and Mayer's carmalum do not fade for > 1 year	- harmful components (hood!), - less suitable for thicker specimens because of loss of volume, - cured resin not soluble in acetone, benzene, ethanol, and xylene	Senior 1970
Caedax, Caedax A, Caedax 547	- neutral, - suitable for thicker specimens because of little loss of volume and drying without gas bubbles, - aniline dyes, azure-eosin, hematoxylin and eosin, Mann's methyl blue with eosin, Nissl thionine, periodic acid-Schiff reaction, and Weigert-Lillie myelin do not fade for 3 months	- harmful components (hood!), - complete dehydration series necessary and time-consuming, - formation of crystals in Caedax with or without phenol (Fig. 15D), may disappear by heating, - drying time > 3 weeks, - Gomori-Burtner methenamine silver staining and Prussian blue fade	Stosch 1952; Lillie *et al.* 1953, p. 69, tab. 1B; Krauter & Rüdt 1980; Göke 2000; this paper
* **Canada balsam**	- - medium lasts > 150 years, - mounting via glacial acetic acid, clove oil, or phenol possible, - suitable for thicker specimens because of little loss of volume and drying without gas bubbles, - cobalt sulfide-alkaline phosphatase method, hematoxylin and eosin, Mann's methyl blue with eosin, periodic acid-Schiff reaction, and Weigert-Lillie myelin do not fade for several months	- harmful components (hood!), - complete dehydration series necessary and time-consuming, - dehydration in ethanol and transfer via xylene or clove oil makes certain taxa brittle, so they break during mounting; these problems may possibly be overcome by dehydration with isopropanol and transfer via *n*-butanol, Cellosolve™, 1,4-dioxane (toxic), Histoclear, or terpineol, - blackening of specimen possible if xylene is replaced by phenol or if potassium hydroxide from maceration remains in specimen, - may mask unstained structures because of high refractive index, - complete drying may require years if not dried on hotplate, - balsam yellows and darkens over time, especially if clove oil is used as clearing agent, - certain stains may be reduced and cationic dyes fade if medium is not neutral, acidity seems to develop *de novo* over time	Kirkpatrick & Lendrum 1941; Salmon 1947; Essig 1948; Gatenby & Beams 1950, pp. 70, 89–91; Lillie *et al.* 1953, tab. 1B; Wagstaffe & Fidler 1955, pp. 173–174; Bender 1967; Barr 1973, p. 16; Berland 1984; Eastop 1985, pp. 248, 269–270; Böck 1989; Huys & Boxshall 1991, p. 451; Brown 1997; Walter & Krantz 2009; this paper
cellulose caprate	- dries within 30 minutes, - good visibility of unstained sections	- harmful components (hood!), - stains like azure-eosin and Prussian blue fade slightly within 15 weeks, Cobalt sulfide fades considerably within 1 month	Lillie & Greco Henson 1955

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
Clarite (= Nevillite V), Clarite X (= Nevillite I)	- does not become acidic and does not discolor over time, - soluble in dioxan, toluene, and xylene, - azure-eosin, hematoxylin and eosin, Masson trichrome, Nissl thionine, periodic acid-Schiff reaction, phosphatase, and Weigert-Lillie myelin do not fade within 3 months	- cavities develop with toluene as solvent but may be avoided by addition of ≤ 10% dibutyl phthalate, - Clarite X becomes slightly yellow over time, - may fade in periphery within 2–3 years, - coverslip may partly detach within 2–3 years, - less suitable for thicker specimens because of loss of volume	Groat 1939; Mohr & Wehrle 1940, 1942; Wicks *et al.* 1946; Lillie et al 1950, 1953, p. 7, tab. 1B
C-M Medium	- specimen can be mounted directly alive and from water, Cellosolve™, ethanol, lactic acid, lactophenol, toluene, or xylene, - no formation of crystals and no visual impact after holding slides at 55°C for 6 months	- specimens cannot be mounted directly from strong acids, strong bases, and glycerol, - heating > 50°C causes darkening of medium	Clark & Morishita 1950; Singer 1967
CMC-9 / CMC-10 (= carboxy methyl cellulose?)	- slides last > 12 months, - specimen can be mounted directly alive and from water and ethanol, - some maceration of inner organs, - works well with stains like acid fuchsin, aniline blue, lignin pink, methylene blue, and pronticil dye (Becker & Heard 1965; McHardy 1966; Mikkelsen 1985)	- less suitable for thicker specimens because of loss of volume and development of cavities, - must be ringed with nail varnish to ensure permanence, - stained specimens cannot be mounted in CMC-10 (Michelson 1960; Davis 1964)	Michelson 1960; Davis 1964; Becker & Heard 1965; McHardy 1966; Mikkelsen 1985
CMCP-9 / CMCP-10 (= carboxy methyl cellulose phenol?)	- specimen can be mounted directly from water, ethanol, glycerol, or solutions containing formaldehyde, - maceration of inner organs	- formation of crystals within few years (Fig. 15G, H), - maceration too intense, - darkens over time, - less suitable for thicker specimens because of loss of volume and development of cavities in the periphery of the coverslip except if ringed, - not suitable for stained specimens, histological sections, and calciferous material, - dries slower than CMC	EMS[1]; Polysciences[3]; Beckett & Lewis 1982; Mikkelsen 1985; Stehr 1987; Roe *et al.* 1991; this paper

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
DePeX / DPX [5] (= Distrene Plasticizer Xylene)	- neutral, - dries quickly, - suited for stains azure-eosin and Mann's methyl-blue with eosin as well as for pyronin G and toluidine blue staining of Araldite sections	- harmful components (hood!), - complete dehydration series necessary and time-consuming, - formation of crystals (Fig. 15I, J), may originate from insufficient de-waxing of mounted sections, - medium may become turbid, - less suitable for thicker specimens because of loss of volume and development of gas bubbles causing specimens to become disrupted, - coverslip may detach over time, may be overcome by cleaning glass surfaces with ethanol before mounting, - refractive index may change leading to optical distortion of specimen, - no drying in an oven or on hotplate to avoid development of gas bubbles - not for cobalt sulfide staining - occasionally unsatisfactory batches of DPX	EMS[1]; Kirkpatrick & Lendrum 1939, 1941; Lillie *et al.* 1953; Krauter & Rüdt 1980, pp. 265–267; Roe *et al.* 1991; Schmolke 1993; Kiernan 1999, 2015; Brown 1997, p. 8; Göke 2000; Ravikumar *et al.* 2014; Sluys pers. com.; this paper
dimethyl hydantoin formaldehyde (DMHF)	- medium lasts > 18 months, - adheres well to glass surfaces, - dries to hard resin, - specimen can be mounted directly from water, acetone, 2-butanone, ethanol, ethyl acetate, formaldehyde solutions, glycerol, lactophenol, methanol, methylene chloride, methyl ethyl ketone, and polythylene glycol, - pH = 6.5–7.5, - maceration of inner organs	- poor contrast with DIC for Copepoda, - aqueous solution: formation of crystals of paraformaldehyde within < 2 years, development of fungi (these problems do not occur in alcoholic solution), - drying < 40°C to avoid formaldehyde to gas out, - not suitable for stains like eosin, methylene blue, and hematoxylin because of rapid fading	Steedmann 1958, 1976c, pp. 189–190; Smith 1966; Bameul 1990; Koomen & Vaupel Klein 1995, pp. 433–434; Richardson 2014, p. 18
Elmer's Washable Clear School Glue™, Colorations™ Washable Clear Glue	- pH more or less neutral, - specimens can be re-mounted by soaking	- not suitable for thicker specimens because of loss of volume	Zander 2014

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
Entellan® new	- dries quickly, - most stains do not fade	- harmful components (hood!), - complete dehydration series necessary and time-consuming, - less suitable for thicker specimens because of loss of volume and development of gas bubbles, - coverslip may detach over time, may be overcome by cleaning glass surfaces with ethanol, - not suitable for stains like carmine, sudan III, toluidine blue, and pyronin G	EMS1; Deutsch 1962; Krauter & Rüdt 1980; Schmolke 1993; Göke 2000
Eukitt™	- medium lasts > 30 years, - specimen can be mounted from acetone, benzene, chloroform, dioxan, ether, isopropyl alcohol, methylbenzoat, terpineol, toluene, and xylene, - dries quickly, - slightly acidic pH, - does not seem to darken over time, - suited for stains like alizarin cyanin RR, alizarin yellow, alizarin viridin, aniline blue, azocarmine, benzo-azurin, Bismarck brown, brilliant Kongo blue, chrysoidine, crystal violet, eosin, fuchsin (rubin S), gentiana violet, hematoxylin, nuclear black, Kernechtrot, Kongo red, light green SF, malachit green, methyl green, methyl violet, methylene blue, naphthazarin, naphthol green B, neutral red, nigrosin, opale blue, orange G, parafuchsin, picric acid, rhodamine, and safranine, - specimen can be re-mounted after several years by soaking in xylene for days to weeks	- harmful components (hood!), - complete dehydration series necessary and time-consuming, - less suitable for thicker specimens because of loss of volume and development of gas bubbles, - coverslip may detach over time, may be overcome by cleaning glass surfaces with ethanol and by coverslip seal, - incomplete polymerization around collagen fibers, - not suitable for stains like carmine, pyronin G, sudan III, and toluidine blue	EMS[1]; Sigma-Aldrich[4]; Schuchert 1999[6]; Ant 1959; Krauter & Rüdt 1980; Frater 1985; Schmolke 1993; Göke 2000; Richardson 2014, p. 17

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
*** Euparal (transparent; green = vert)**	- medium lasts > 50 years, - mounting from 80% ethanol possible, - does not mask unstained structures, - does not yellow over time, - does not become brittle over time, - refractive index favorable over that of Canada balsam (for Diptera and other Insecta), - suitable for thicker specimens because of little loss of volume and drying without gas bubbles, - aniline blue, azure-eosin, Nissl thionine, and Weigert-Lillie myelin fast method do not fade for several months, suitable for Romanowsky stain, - green formula for better contrast of hematoxylin staining, - soluble in 95% ethanol for re-mounting after years	- harmful components (hood!), - dehydration series necessary and time-consuming, - dehydration with ethanol and transfer via Euparal Essence makes certain taxa brittle, so they break during mounting; these problems may possibly be overcome by dehydration with isopropanol and transfer via Cellosolve™ or Histoclear, - Feulgen stain fades - Euparal from ASCO (GB) more satisfying than from Chroma (Germany) but no arguments provided	Imms 1929; Mohr & Wehrle 1940; Salmon 1947; Lillie *et al.* 1953: tab. 1B; Wagstaffe & Fidler 1955: p. 175; Dewse & Potter 1975; Berlin & Miller 1980; Garner & Horie 1984; Freeman 1987; Böck 1989; Huys & Boxshall1991: p. 451; Koomen & Vopel Klein 1995; Brown 1997: pp. 5, 8; Brown & Boise 2005, 2006; Sørensen & Pardos 2008; Neuhaus & Sørensen 2013; Neuhaus 2013
Fluoromount G™	- specimen can be mounted directly from water, - soluble in water for re-mounting after about 10 years,	- formations of cavities within 10 years (Fig. 20A– E), - formation of crystals within 10 years (Fig. 20A–E), - segregation of components of medium (Fig. 20F), - hygroscopic	EMS1; Spurr 1954; Witte 1976; Neuhaus 2013, 2017, this paper
glycerol-gelatin (Kaiser's)	- specimen can be mounted directly from water or glycerol (preferably in order to avoid cracking of medium), - specimens may last 54 years, - heating allows re-mounting	- ringing supposed to prevent evaporation of water, - mounting specimens at too high temperature destroys medium, - glycerol may separate from gelatin and coverslip detaches, - formation of cavities and cracking coverslip (Fig. 18A), - occasionally formation of cracks in medium	Gatenby & Beams 1950, p. 209; Evens 1961; Adam & Czihak 1964, pp. 162, 291–292; Steedman1976c, pp. 190–191; Martin 1978, p. 105; Hevers 1985; Sanderson 1994, pp. 154–155; this paper
glycerol-gelatin (Zirkle's aceto-carmin)	- medium fixes, stains, and mounts in a one-step application	- high osmotic value of glycerol may lead to collapse of delicate structures, - dextrose makes medium hard and brittle, - un-ringed mounts deteriorate under humid conditions, - formation of peripheral crystals, - fixation by glacial acetic acid not satisfying for fine details	Zirkle 1937, 1940

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
***glycerol-paraffin mount sealed with Glyceel; *double-coverslip glycerol mount in Canada balsam or Euparal**	- medium lasts "indefinitely", - mounting via glycerol-ethanol mixture, so no harmful components used, - specimen can be manipulated on hotplate any time, - "clearing" of specimen because of similar refractive index as proteins, - does not mask unstained structures, - does not form cavities or crystals over time, - does not blacken over time, - specimen can be re-mounted easily	- glycerol may leak, - glycerol used for mounting must be kept anhydrous in oven (40°C) or desiccator with silica gel, - coverslip seal and regular control inevitable	Diehl 1929; Myers 1936; Adam & Czihak 1964; Travis 1968; Kohlmeyer & Kohlmeyer 1972; Taft 1983; Hooper 1986a; Maybury *et al.* 1991; Morse 1992; Hooper *et al.* 2005; Sudhaus pers. com.; this paper
gum-chloral media like Berlese's and Hoyer's mounting medium (see also Tab. 7)	- specimen can be mounted alive or directly from water, ethanol, and formaldehyde, - maceration of inner organs results in excellent quality of cuticle, - favorable refractive index, - iodine stain provides higher contrast, - acetic acid (if included in recipe) leads to expanded appendages of arthropods, - some (!) specimens may last 40–60 years, - water-solubility allows re-mounting	- chloral hydrate has sedative properties, is regulated now at least in the USA, and for this reason may be difficult to obtain, - delicate plant specimens may collapse if medium is not added gradually, which is time-consuming, - formation of cavities within < 10 years, - formation of crystals within < 10 years, - maceration too intense, depending on amount of chloral hydrate and time, - segregation of components of medium, - fine granulation within months or years, - blackening reported	Swan 1936; Mitchell & Cook 1952; Evans & Browning 1955; Ossiannilsson 1958; Rusek 1975; Higgins 1977, 1982, 1983; Zander 1983; Eastop 1985; Noyes & Polaszek 1989; Upton 1993; Halliday 1994; Koomen & Vaupel Klein 1995, p. 429; Amrine & Manson 1996; Brown 1997; Faraji & Bakker 2008; Neuhaus 2013, fig. 5.5.2C; Villani *et al.* 2013; Amrine, pers. com; this paper
modified gum-chloral medium with BTDA	- no granulation of medium	- formation of crystals, - maceration too intense, - partly loss of autofluorescence signal of chitin in the cuticle of Acari for confocal laser scanning microscopy after > 1 year	Chetverikov 2012; Amrine pers. com.
lactophenol gel	- specimen can be mounted from water, - rapid mounting, - delicate specimens do not collapse, - macerates specimens, - ingredients of medium easily obtainable	- mount does not harden entirely, - mount must be ringed in order to prevent collapse of a delicate plant specimen in the periphery of the coverslip	Zander 1983

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
Loctite® UV 357	- low viscosity, - low toxicity, - fast curing with UV-light within 30 seconds, - high clarity, - non-fluorescent, - no fading and translocation of dyes, no detachment of coverslip, no formation of crystals within 2 years	- medium cannot be dissolved again once cured, - mounting must be done away from direct daylight, because sunlight will cure medium quickly even through closed windows	Denton 1987
Loctite® 363™ Impruv® Potting Compound Light Cure	- forms thinner film than solvent-based media, - polymerization within 10 s with UV light, - suitable for thicker specimens because of little loss of volume during curing, - no immediate fading of cresyl violet, hematoxylin and eosin, silver stains, and thionin, - no immediate fluorescence of medium, - re-mounting possible at least within months by soaking slide in methylene chloride overnight	- harmful components (hood!)	Silverman 1986
Malinol	- suitable for thicker specimens because of little loss of volume and drying without gas bubbles	- harmful components (hood!), - complete dehydration series necessary and time-consuming	Krauter & Rüdt 1980
Naphrax™	- no fading of stains within 10 years (hematoxylin and eosin, methylene blue and eosin, and Wright's stain), - high refractive index is useful for diatoms	- harmful components (hood!), - darkening after about 50 years	Fleming 1943, 1954; Brown 1997, fig. 25
Permount™ (old: formula before 1953)	- azure-eosin, hematoxylin and eosin, Mann's methyl blue with eosin, Masson's trichrome stain with aniline blue, Nissl thionine, periodic acid-Schiff reaction, Van Gieson's picric acid with acid fuchsin, Weigert-Lillie myelin, and Ziehl Neelsen acid-fast method do not fade for several months	- basophilic stains fade	Lillie *et al.* 1953, p. 66, tab. 1B
Permount™ (new: formula after 1953)		- harmful components (hood!), - formation of annual bands of cracks within 5 (or 20) years (Figs 16D–J, 17A–F), - re-mounting supposedly possible by soaking in xylene, but spines may break or get lost at least in Kinorhyncha	EMS1; E. Metzler in Brown 1997, p. 9; Hollander & Frost 1970, 1971; Loveland & Centifanto 1986, p. 189; Roe *et al.* 1991; Wiggins & Drummond 2007, p. 8; this paper
Piccolyte®	- favorable refractive index, - good adhesion to glass	- similar resin: yellows within few years	Wicks *et al.* 1946; Lillie *et al.* 1950, p. 2

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TABLE 6. (Continued)

(Trade) name	Advantages	Disadvantages	References
polyvinyl lactophenol	- specimen can be mounted directly alive or from water, acetone, ethanol, glycerol, acetic acid, formaldehyde, glycerol, lactic acid, and methyl salicylate, - clearing of morphological structures, - maceration of inner organs, - refractive index favorable over Canada balsam and gum-chloral media, - water-solubility seems to allow re-mounting despite some polymerization over time (ability for re-mounting is doubted by Singer 1967)	- less suitable for thicker specimens because of loss of volume, - formation of cavities (Figs 18B–H, 19A–D), - formation of granular precipitations, - formation of crystals (Figs 18B–H, 19A–D), - segregation of components of medium, - fading of alkaline stains, - maceration too intense within < 10 years, - medium may become turbid, possibly because of water from specimen, - shriveling of specimen because of phenol, - coverslip seal necessary	Salmon 1951a, 1954; Heinze 1952; Mitchell & Cook 1952; Lipovsky 1953; Beer 1954, p. 1109; Evans & Browning 1955; Ossiannilsson 1958, p. 2; Evans *et al.* 1961, p. 81; Ribeiro 1962; Singer 1967, pp. 480–481; Bink 1979, p. 160; Woelke & Göke 1984, figs 1–3; Bartsch 1988, p. 419; Wilkey 1990, p. 347; Huys & Boxshall 1991, p. 451; Koomen & Vaupel Klein 1995, pp. 434–435; Mills & White 1999, p. 132; Neuhaus 2013, p. 278, figs 5.5.1.C–D, 5.5.2.A–B; this paper
Polyviol	- medium lasts > 20 years, - specimen can be mounted directly from water, - maceration of inner organs	- formation of cavities (Fig. 19E, F), - specimens shrink if treated with lactic acid or lactophenol before mounting	Rusek 1975; Danielsson 1985; this paper
Styrax	- high refractive index is useful for diatoms	- harmful components (hood!), - drying time about 4 weeks, - turns yellow on heating during mounting process	Göke 2000
Technovit® 7100 and hardener 2	- medium water-soluble, - serial sections possible if medium with plasticizer, - histological sections may be very thin (2–5 µm) resulting in high resolution imaging of tissue, - harder plant material tissues may be sectioned, - temperature does not exceed 40°C during mounting, - suitable for stains basic blue/ alum-kernechtrot, Giemsa's stain, Lugol's iodine, ruthenium red, and toluidine blue	- harmful components (hood!), - no dissolution in organic solvents once hardened, - stains like hematoxylin and safranin may also stain matrix, - medium does not attach well to cuticle of plants	Gerrits & Smid 1983; Vermathen 1993
Venetian turpentine medium (Wilson)	- aceto-carmine stain of plant material lasts > 3 years, - Feulgen stain of plant material and invertebrates lasts > 3 months	- harmful components (hood!), - stain may fade in the tropics, - medium may dry out	Margolena & Barsony 1931; Wilson 1945; Haunold 1968
Water-glass-Glycerol	- specimen can be mounted from water or glycerol, - suitable for basic stains like basic fuchsin and safranin O because of basic pH of medium, - slides last > several months (Creaser & Clench 1923)	- not suitable for stains like methyl green, orcein, and toluidine blue, - less suitable for thicker specimens because of loss of volume (due to evaporation of water) and development of cavities	Creaser & Clench 1923; Zander 2014

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TABLE 6. (Continued)

[1] EMS: = Electron Microscopy Sciences, Available from: http://www.emsdiasum.com/microscopy/products/histology/mounting_media.aspx (accessed 21 May 2015).
[2] Merck: Available from: http://www.merckmillipore.com/DE/de/product/Aquatex%C2%AE,MDA_CHEM-108562#anchor_BRO (accessed 21 May 2015).
[3] Polysciences Inc., technical data sheet 432, rev. 17.X.2011
[4] Sigma-Aldrich: Available from: http://www.sigmaaldrich.com/catalog/product/fluka/03989?lang=de&region=DE (accessed 21 May 2015).
[5] DePeX and DPX seem to differ only in the "relative proportions of polystyrene and dibutyl phthalate" (Roe *et al.*, 1991).
[6] Schuchert 1999: Available from: http://taxacom.markmail.org/search/?q=slide%20restoration#query:slide%20restoration+page:1+mid:42qqzw5gzhlz7n6c+state:results (accessed 07 April 2016).

TABLE 7. Observations and arguments favoring or rejecting the use of gum-chloral media, except Viscol, which lacks chloral hydrate (Dahl 1951), based on selected references.

Advantages	Disadvantages	Reference
- permanence of gum-chloral mounts with glycerol better than of those made with glycerol-gelatin, - convenient application, no coverslip seal necessary, - favorable refractive index	- fungi may develop within 6 months in the storage bottle but can be removed by addition of more chloral hydrate and subsequent filtering	Hoyer 1882, pp. 23–24
- medium allows "killing, fixing and mounting in a single operation" and "in an extended condition", - specimens do not shrink or distort, - ringing of Faure's fluid not necessary "under English conditions", - slides last at least 10 years	- "in some cases very slight distension occurs", - ringing of Faure's medium may be necessary under tropical conditions, ringing of Berlese's medium is required	Imms 1929
- "low refractive index of Berlese's fluid permits of extremely clear [...] images of transparent objects", - maceration of inner organs within about one day, - acetic acid leads to extpanded appendages, - "The keeping quality of mounts appears to be very satisfactory; and the mounts for most purposes are permanent. I have seen slides about ten years old which, except for cases of crystallization, show no signs of deterioration", - "mount under Australian conditions is permanent without ringing"	- formation of "delicate needle-shaped crystals" in Imm's medium because of "excessive" amount of chloral hydrate and evaporation of water, "aided by falling temperature" (consequently, Swan reduces the amount of chloral hydrate in his recipe; crystals can be dissolved again by glacial acetic acid and heating the mount), - Berlese's medium not suitable for stained specimens	Swan 1936
- Berlese's mixture represents a combined macerating and mounting medium, - favorable refractive index of Ewing's medium for mites because of reduced amount of glycerol and chloral hydrate, - better physical propertiesof Ewing's medium by addition of glucose syrup, - Ewing's medium more durable than Berlese's medium	- crystallization because of too much chloral hydrate, substitution of part of chloral hydrate by water, - no heating in an oven > 33°C for permanent mount	Ewing 1939, pp. 2–3

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
- specimens can be mounted directly alive or from glacial acetic acid, lactic acid, and water, - favorable low refractive index, - "quickness of clearing and mounting", - in Womersley's medium, crystallization is supposed to be prevented by substituting lactic acid and phenol in the medium for acetic acid, - mounts may be re-mounted by dissolution in water	- Berlese's and Faure's medium form crystals, this "can be corrected by running a little acid around the edge of the cover slip and gently warming", - formation of crystals in Berlese's, Faure's, and Imm's medium because of evaporation of acetic acid, - Womersley's medium darkens over time on exposure to light because of the phenol in the medium	Womersley 1939, pp. 291–292, 1943
- specimen may be mounted dry, alive, from ethanol or formaldehyde, - Doetschman's medium allows fixing, dehydrating, staining, macerating, and mounting in one step, - stains like basic fuchsin, alcoholic fast green, and picric acid can be incorporated in medium	- reduction of water and chloral hydrate as well as addition of glycerol and a stain avoid crystallization, - mount will be permanent if kept at 37°C overnight or at 50°C for one hour	Doetschman 1944
	- formation of crystals in Berlese's medium will destroy specimen	Salmon 1947
	- sealing "seems not to be necessary in normal circumstances", - Murrayite supposed to cause fine granulation of gum-chloral medium	Lambers 1950
	- in Viscol, crystals with lactic acid form "probably due to the calcium and magnesium ions" from gum arabic, - "greatest danger is the contraction of the medium if much water is permitted to evaporate", which leads to crystallization	Dahl 1951
	- formation of crystals in Berlese's and Faure's medium, - fungal growth	Heinze 1952
- specimen may be mounted in Hoyer's medium alive or preserved, - satisfying "optical properties", - maceration of inner organs	- less suitable for thicker specimens because of loss of volume and crushing of specimen, ringing prevents this	Mitchell & Cook 1952
	- glycerol is supposed "to prevent over-drying and cracking" of gum-chloral media and to maintain "plasticity and adhesiveness" to glass surfaces, - sugars raise the refractive index of gum-chloral media; experimental slides with high concentrations of glucose, invert sugar, maltose, sucrose, and white Karo crystallize within a month	Lillie *et al.* 1953, pp. 73–75

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
- re-mounting easy by soaking in warm water	- most Berlese-type media "crystallize in a relatively short time" first near the edge of the coverslip, media probably non-permanent	Beer 1954, pp. 1109–1110
- specimens can be mounted directly alive or from ethanol	- specimens in Faure's and Hoyer's medium "tend to over-clear and suffer damage due to shrinkage over a period of years", - mount should be ringed with Euparal or gold-size to prevent shrinkage of medium	Evans & Browning 1955
- "excellent penetrating properties and clears well", - medium with reduced amount of chloral hydrate used "with considerable success for some years"	- Berlese's medium crystallizes out "when set, or at low temperatures" , - ring with Glyceel	Wagstaffe & Fidler 1955, p. 175
	- Berlese's and Faure's medium reveal fine granulation within months or years, - "sealing is necessary to prevent air creeping into the mounts from the edges of the cover-glass"	Ossiannilsson 1958
	- Berlese's medium does not harden sufficiently, - exposure to lower temperatures harmful, - formation of cavities, - unfavorable refractive index, - specimen macerated too much, organs may shrink	Hirschmann & Woelke 1960
	- "unstable in humid climates", - cavities develop from periphery of coverslip, even if coverslip seal exists	Richards 1964
- mount prepared simpler and faster in Hoyer's medium than in Canada balsam and Euparal, - less distortion of specimens than in Canada balsam and Euparal, - refractive index more favorable, - re-mounting easy because of water-soluble medium	- peripheral cavities develop on drying of the medium, - uptake of moisture in humid climates if un-ringed	Quate & Steffan 1966
- mount prepared simpler and faster in gum-chloral media than in C-M Medium, polyvinyl lactophenol, glycerol-jelly, glycerol, and lactic acid, - refractive index more favorable (less distortion) if studied with phase contrast, - re-mounting easy because of water-soluble medium, - conclusion: Andre's medium "is one of the most practical media currently available to the acarologist"	- specimens must be washed thoroughly after maceration "otherwise lactic acid and phenol crystals will develop during curing of preparation", - "slight crystallization of the chloral hydrate component is not uncommon", problem can be avoided by reducing the amount of chloral hydrate to 100–150 g, - < 100 g chloral hydrate will cause specimen to shrivel, - "Oven drying causes the coverslip to assume a buckled, convex shape, due to an unequal drying of the medium at the edges of the coverslip. These stresses later tend to rupture ringing compounds and draw air under the coverslip."	Singer 1967

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
	- formation of crystals as Berlese's or Faure's medium dry, because "too high proportion of chloral hydrate", - replacement of glycerol by glucose syrup and acetic acid "reduces risk of granulation" and of turning opalaescent, - permanent slides should be ringed with Canada balsam within 112 months	Eastop & Emden 1972, pp. 7–8
- Hoyer's does "not need to be ringed if adequate mountant is used", - lasts at least 15–20 years, a "miracle mounting fluid", - "powerful clearing agent" (actually: macerating)	- "extremely thin mounts [...] may dry out eventually if not ringed", - removes several stains	Cunningham 1972, pp. 906–910
- chloral hydrate removes "opaque soft tissue", - "chloral hydrate softens, expands, and plasticizes exoskeletons of delicate arthropods" and "displays structural features", - gum arabic does not collapse on drying and does not crush a specimen, - gum arabic gives "substance to the medium", helps "to become sufficiently viscous upon losing some water", and "tends to resist humidity in the atmosphere", - phenol in the medium clears specimen, - glycerol prevents excessive hardening of specimen	- sorbitol "holds chloral hydrate in solution" along with glycerol and prevents its recrystallization, - sorbitol and formaldehyde prevent migration of the stain iodine from specimen into medium, - chloral hydrate dissolves inner organs too much over time, - formaldehyde "slows the dissolving action of chloral hydrate on chitin", - slides are more permanent if ringed with excess Hoyer's medium and if amount of water is reduced; a non-water-soluble coverslip seal will keep iodine-potassium iodide in mount	Jeppson *et al.* 1975, pp. 117, 387–388
- specimens in Swan's medium (probably Puri's according to Upton 1993) may last 50–60 years	- formation of crystals in unsuitable modifications of Swan's medium (Upton 1993: Puri's medium)	Rusek 1975
- no "preliminary fixing operations as required for resin mounts", - keeping slide at 45°C for 2–7 days and than for 7 days at room temperature plus ringing makes "the preparation more or less permanent", - "Properly mounted and ringed slides may be expected to last indefinitely."	- Hoyer's medium is hygroscopic and "subject to displacement of medium by atmospheric moisture, and to eventual crystallization and breakdown", - "more assured permanency of slide preparations may be possible by using oil soluble resins or by employing a double coverslip technique"	Krantz 1978, pp. 88–91
- Hoyer's with favorable refractive index, - "convenient, practical, and permanent", - mites can be mounted alive and directly from water, ethanol, and Nesbitt's fluid (chloral hydrate, hydrochloric acid, water)	- ringing prevents evaporation and uptake of humidity causing formation of peripheral cavities and makes mounts permanent, - if ring fails, medium dries out and deteriorates, - specimens macerated "in lactic acid or lactophenol should be washed [...] to avoid causing crystallization"	Martin 1978, pp. 108–109, 135
- Faure's medium water-miscible, - macerates specimen well	- hygroscopic medium becomes sticky in humid climates, so ringing is recommended	Bink 1979

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
- specimen can be mounted in Hoyer's medium directly from a mixture of glacial acetic acid and lactophenol or chloralphenol, - better resolution of structures with phase contrast in Hoyer's medium than in Canada balsam, - re-mounting easy because of water-soluble medium	- ringing with Glyceel necessary "to insure a permanent mount" and to prevent drying out of the mount, - Canada balsam "is a much more permanent mounting medium than Hoyer's and does not require ringing"	Rosen & DeBach 1979, pp. 15–16
- no dehydration of specimen before mounting in Berlese's medium, - no discoloration "over long periods", - "specimens do not appear to become brittle"	- "excessive glucose may cause crystallisation", - specimens fixed in any fixative other than 70% ethanol should "be washed in distilled water to prevent precipitation of gum arabic on the specimen surface"	Henshaw 1981
	- un-ringed Berlese's, Faure's, and Hoyer's medium shrink on drying within about 40 years, so coverslip crushes specimen; specimen will fragment on soaking in water for re-mounting, so re-mounting is impossible, - consequently, (potential) primary type material should not be mounted in a water-soluble medium, - using phase contrast will overcome visibility problems of Canada balsam	Noyes 1982
- mounts in Hoyer's medium are prepared rapidly, - "plant material is cleared well", - favorable refractive index, - "preparation is solid and sturdy", - "If glycerol is present in sufficient quantity to offset atmospheric dryness, luting is unnecessary and the mounts are considered essentially permanent."	- delicate plant specimens may collapse if medium is not added gradually, which is time-consuming, - chloral hydrate is regulated in the USA and for this reason is difficult to obtain	Zander 1983
	- "a submicroscopic granulation appears after years" in Berlese's and Faure's medium, - "grape-sugar [...] sometimes crystalizes in the mounts", - specimens may disappear after 20 years because of KOH in combination with chloral hydrate	Danielsson 1985
- Berlese's and Faure's medium are water-miscible	- some slides may last > 40 years, others deteriorate	Eastop 1985
	- specimens in Hoyer's are "rapidly disintegrating", - "I have grave doubts that ringing will really make these slides permanent, mite workers continue to insist that a properly ringed mount will last"	Schauff 1985
	- formation of crystals in Swan's medium even if sealed with Euparal, possibly because of variable quality of gum arabic sampled from different trees	Freeman 1987

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
	- "Hoyer's is hygroscopic. Under humid conditions cover slips will loosen and slip unless the mount is carefully ringed."	Stehr 1987, p. 16
- "unrivalled [...] for small insects", "excellent medium", "none to match up to Berlese Fluid" - ringing with Glyceel or nail varnish recommended	- glucose > 8% generally causes crystallization, - ringing with Euparal causes discoloration of medium, - discoloration because of "residual preservatives such as phenolics and formaldehyde", - "Gum Arabic of different origins may produce slightly different results"	Disney & Henshaw 1988
- "The loss of resolution, resulting from mounting in Canada Balsam, is overcome by the use of phase-contrast lightning. While the latter is a luxury not available to many, the gain in the reliability of species recognitions is, perhaps, to be preferred to the wider user-friendliness of identification keys that do not assume phase-contrast will be used.", - inner organs remain preserved in Berlese's medium, - mounting takes much shorter than in Canada balsam, - re-mounting easy because of water-soluble medium	- "permanence [...] is not necessarily the primary consideration", - "For many groups of small insects the risk of loss of permanence, resulting from the use of Berlese Fluid, is offset by many advantages. If an occasional type specimen needs remounting after 50 or 100 years, that seems a small penalty."	Disney 1989
- some mounts in Berlese's and Hoyer's medium may last 50 years	- primary type material should not be mounted in a water-soluble medium, because many specimens will be crushed by the coverslip on drying of the medium, - deterioration occurs even if ringed properly	Noyes & Polaszek 1989
	- plant material loses its color in Hoyer's medium, - "delicate cell walls tend to shrink and distort"	Frahm 1990
	- "permanence of such Berlese-mounts depends both on the quality of the seal that has to prevent desiccation and shrinking of the mountant, as well as on the thickness of the preparation" (specimen thinner than 20 µm)	Koomen & Vaupel Klein 1995
- Reyne's fluid dries quickly, - suitable refractive index for unstained Crustacea, - macerates internal organs	- ringing required for "truly permanent mounts"	Stock & Vaupel Klein 1996
	- peripheral cavities develop on drying of Berlese's or Faure's medium, - oxidative discoloration of un-ringed medium	Notton 1995
	- Berlese's and Hoyer's medium: "slides are rarely permanent", specimens "become opaque, shriveled or too cleared"	Amrine & Manson 1996

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TABLE 7. (Continued)

Advantages	Disadvantages	Reference
	- gum-chloral media may show "phenol blackening with pink, bluish or black areas emanating from the specimen", - blackening assumed to originate from reaction of gum-chloral medium with Euparal used for ringing or from insufficient washing of macerating and clearing agents, - chloral hydrate supposed to start crystallizing in the periphery of the coverslip, because water evaporates due to the failure of the coverslip seal	Brown & Boise 2005, p. 27
	- "The sealant had failed and allowed the Berlese to dehydrate, thus causing the formation of Chloral-hydrate crystals throughout the mount."	Gunter & Brown 2005
- gum-chloral media "are preferred because of their ease of use and their excellent optical properties", - re-mounting easy because of water-soluble medium	- "Water-miscible media built around gum arabic may produce poor results when improperly formulated [...], but these essentially semipermanent mountants are preferred because of their ease of use and their excellent optical properties.", - "Hoyer's medium slide preparations have a greater tendency to spoil than do slides with nonaqueous media", - "preparations that are not reprocessed may eventually crystallize or deteriorate, and the medium becomes filled with oily globules or takes on an opaque, granular appearance", cause is probably "an interaction between the medium and some contaminants in the clearing agent or sealant, or in the mite itself" - mount must be ringed to prevent "water from entering or leaving the mount", - "Properly mounted and ringed Hoyer's medium slides may be expected to last for decades, while a poor preparation will spoil in a few months."	Walter & Krantz 2009, pp. 90–91
- Hoyer's medium: "ringed (sealed) preparations are permanent, with a storage record of more than 40 years"		Criado-Fornelio *et al.* 2014

Also, unusual media like glucose dissolved in formaldehyde have been tested and are supposed to last longer than 10 years, although the coverslips are not sealed (Taft 1978). It can be expected that the formaldehyde will have diffused out of the mount within a short time because of its volatility (Tab. 10). This will also apply to a mixture of Karo syrup and formaldehyde (Webb 1997). Zirkle (1940) described a range of water-soluble fixing, staining, macerating, and mounting media as a one-step application with carmine for plant material. His hydrocarbon-soluble media, especially Venetian turpentine but also Canada balsam and sandarac, with carmine allow fixing, staining, dehydrating, clearing, macerating, and mounting in one step if an emulsifier composed of acetic acid, phenol, and propionic acid is used. Ethylenediamine functions to keep ferric nitrate in solution, which is necessary for an intensive carmine staining (Zirkle 1940). Doetschman (1944) recommended a one-step fixing, dehydrating, macerating, staining, and mounting gum-chloral medium, which is actually the case with all gum-chloral media except for the staining property (Tab. 7). Shortcoming of imported Canada balsam and cedar oil encouraged replacement of these media by natural resins from various species of *Pinus* in former Yugoslavia (Varićak & Pejoski 1951). General information about synthetic polymers and about the mechanisms of their deterioration by weathering was summarized by Wypych (2012, 2013).

In this paper, all mounting media and coverslip seals, which consist of organic components and solidify, are regarded as polymers; their ingredients are listed in a way to figure out their potential function at a glance (Tabs 5, 8). Table 5 also contains the liquid glycerol, because mounts in it are fully reversible. It should be kept in mind that the exact composition of all commercial mounting media and coverslip seals is unknown and may change over time (Tabs 5, 8; see chapter 2. Materials and methods). Also, volatile components may evaporate over time and may not be detectable with chemical analysis such as Raman spectroscopy (Schmid *et al.* 2016). Table 10 lists such volatile ingredients, for which vapor pressure data are available readily.

3.7.2 Permeability of polymers for gases and vapors

Generally, polymers are permeable to some degree for various gases and vapors like water and oxygen; such data is especially interesting for packaging of food and of electronic devices, controlled drug release in medical applications, and water desalination and available for many synthetic and some natural polymers (Tab. 11; Traeger 1976; Hagenmaier & Shaw 1991; Pauly 1999; Gerlach *et al.* 2001; Stern & Fried 2007; Wypych 2012; Pethe & Joshi 2013). The permeability coefficient P of a polymer is defined as: P = (volume of permeating gas) x (effective polymer membrane thickness) x $(\text{time})^{-1}$ x $(\text{polymer membrane area})^{-1}$ x $(\text{pressure difference across polymer membrane})^{-1}$; the dimension used in this article is $P = 10^{-12}$ x cm^3 x cm x s^{-1} x cm^{-2} x Pa^{-1} at 25°C (Tab. 11; Pauly 1999; Stern & Fried 2007, p. 1038; Wypych 2012). Unfortunately, data about the permeability of polymers for gases and vapors is provided in the literature in different units, and the exponent of ten is given sometimes with a positive and sometimes with a negative value, even within one and the same publication (Pauly 1999; Wypych 2012 and references cited therein). Reading Traeger (1976) and Gerlach *et al.* (2001) carefully, the exponent of ten should have a negative value for polymers, and this is corrected in Table 11. It has to be kept in mind that the data provided in Table 11 for homopolymers has to be regarded as only a rough estimation of the order of magnitude of permeability of mounting media and coverslip seals, which are composed of a variety of ingredients (Tabs 5, 8). Also, mixtures of gases like in air may behave slightly different than pure gases; a high humidity may increase permeability for other gases, because the water may plasticize the polymer to some degree (Comyn 1986, p. 319). The permeability may be reduced by a large amount of filler and in more cross-linked resins in comparison to linear polymers, and it will be increased by a plasticizer (Comyn 1986, pp. 317–318; Pauly 1999; Gerlach *et al.* 2001).

Considering the extensive discussion about the impact of a coverslip seal on the durability of a slide mounted in a gum-chloral medium (Tab. 7), we find it useful to calculate the time required for gaseous water molecules to permeate a synthetic resin until reaching the specimen. Assuming a slide with a circular coverslip of 15 mm diameter, a mount 0.1 mm thick, poly(methyl methacrylate) as mounting medium with P = 48–190 x 10^{-12} cm^2 x s^{-1} x Pa^{-1} (Tab. 11), a surface area of (2 x π x 7.5 mm radius of coverslip) width x 0.1 mm height of mount, a pressure difference between the center of the mount and the environment of 3,167 Pa at a relative humidity of 100% and at 25°C (1,583.5 Pa at RH = 50%), a volume of 0.1 mm^3 (= 1 mm x 1 mm x 0.1 mm) water vapor would require 0.7–3 hours (1.5–6 hours at RH = 50%) to diffuse from the periphery of the coverslip to the center penetrating a

specimen of 1 mm diameter totally. If the same slides is sealed with shellac as a coverslip seal 1 mm thick and P = 0.0463 x 10^{-12} cm^2 x s^{-1} x Pa^{-1} (Tab. 11), a surface area of (2 x π x 7.5 mm radius of coverslip) width x 1 mm thickness of seal, a pressure difference between the center of the mount and the environment of 3,167 Pa at a relative humidity of 100% (1,583.5 Pa at RH = 50%), a volume of 0.1 mm^3 water vapor would penetrate the seal around the coverslip within 17 days (33 days at RH = 50%). This means that shellac as a coverslip seal would offer an additional protection of the slide of 17 days (33 days at RH = 50%). Among the polymers listed in Table 11, shellac belongs to those resins with the lowest permeability for water. These calculations roughly show that both a polymer and an additional coverslip seal do not really represent a permanent barrier for water molecules for decades. This finding agrees with a more general statement for the time necessary to penetrate a polymer of about 10 mm thickness indicating days rather than months and years (Traeger 1976, fig. 1).

3.7.3 Refractive index of mounting media

Unstained specimens are best investigated by transmitted bright field microscopy if the refractive index of the mounting medium differs most from that of the specimen (Marshall 1932; Dahl 1951; Lillie *et al.* 1953, p. 57; Adam & Czihak 1964, p. 167; Singer 1967, p. 478; Krauter & Rüdt 1980, pp. 264–265; however, for recent software development see chapter 3.12.1 Microscope equipment), whereas stained histological sections would benefit from a mounting medium with a refractive index close to that of glass (Adam & Czihak 1964, p. 167) or that of the specimen (see also chapter 3.1.3 Physical clearing; Groat 1940; Gatenby & Beams 1950, p. 205; Lillie *et al.* 1953, p. 57; Singer 1967, p. 478; Bradbury & Evenett 1996, p. 43; Ravikumar *et al.* 2014). A difference between the refractive indices of the specimen and of the mounting medium of DnD = 0.02 results in a low contrast, a DnD = 0.05 prompts a moderate contrast, a DnD = 0.1 causes a high contrast, and a DnD = 0.2 ensures a very high contrast (White 1970, p. 259). Most biological structures labelled with antibodies and fluorescent probes and studied with confocal laser scanning microscopy profit from a similar refractive index of both the immersion oil, coverslip, mounting medium, and specimen resulting in a mismatch of refractive indices as small as possible (Cody *et al.* 2005). If the refractive index of the mounting medium is closer to that of the specimen in thicker mounts, more heavily sclerotized structures stand out more clearly in transmitted light microscopy, whereas more delicate structures may become less visible; if the index of the medium differs more from that of the specimen, more heavily sclerotized structures may partly obscure neighboring more delicate structures (Singer 1967).

Certain details of diatoms, helminths, kinorhynchs, and mites are partly masked by Canada balsam (Fleming 1943; Barr 1973; Berland 1984; Brown 1997), but are considerably better visible in glycerol, lactic acid, and glycerol-gelatin because of the different refractive indices of the different mounting media (Tab. 6; Berland 1984; Neuhaus pers. obs.). The refractive index varies in a given mounting medium depending on the temperature of the mounting medium and on the amount of solvent still in the medium. Also, the size of a specimen varies slightly if mounted in different mounting media of different refractive indices (Skene 1969). For positive phase contrast (Crossmon 1949) and for bright field microscopy (Skene 1969), these authors recommend using a mounting medium with a slightly lower refractive index than that of the morphological character of the specimen. The refractive index of a specimen may be determined by temporarily mounting a specimen in different media with different indices (Crossmon 1949). A specimen shows less contrast in a medium of a lower refractive index than that of glass in comparison with a medium of higher index than that of glass, because in the medium with a higher index the "rays of light [...] will be further refracted towards the axis in passing from the ordinary glass slide into the medium" (Marshall 1932, p. 277; Roe *et al.* 1991). Staining a specimens and observation with phase contrast or differential interference contrast optics may partly overcome problems based on an insufficient refractive index of the mounting medium (Noyes 1982; Disney 1989; Noyes & Polaszek 1989). Hirsch (1953) suggests a refractive index of nD = 1.515 for the study of stained sections with phase contrast and recommends mounting the sections in cedarwood oil, which hardens over time.

TABLE 8. (Potential) contents of selected coverslip seals.

Name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Preservative	Filler	Remarks	References
Araldite		pre-polymer bisphenol A epoxy resin, hardener butylphenyl glycidyl ether	dibutyl phthalate			accelerator	Fig. 14; Adam & Czihak 1964; Boparai & Chhabra 1968; Horie 2011, pp. 290, 296
asphaltum	benzene or oil of turpentine	asphaltum, polymerized linseed oil, James 1887: compounds of gum amber			yellow lead oxide (= litharge, PbO)	commercial "Brunswick black" in addition with lamp black, india rubber, and naphtha; Gray 1954: some recipes with goldsize	James 1887, pp. 62–63; Behrens 1892, p. 71; Spence 1940b, pp. 141–142; Gray 1954, pp. 652–656; Bracegirdle 1978, p. 94
Brunswick black	partly volatile components of Venetian turpentine (see Tab. 5)	compounds of Venetian turpentine (see Tab. 5), Trinidad asphaltum, partially polymerized and oxidized linseed oil				other recipes: also with yellow lead oxide (PbO) or with rubber or with goldsize and Canada balsam	Figs 10, 11; Gray 1954, pp. 652–653
Canada balsam						see Tab. 5	
caoutchouc cement	chloroform, partly volatile components of gum mastic (anethole, α-campholene aldehyde, caryophyllene, *o*-cresol methyl ether, *p*-cymene, limonene, linalool, α-pinene, β-pinene, sabinene, terpineol)	caoutchouc, compounds of gum mastic (β-amyrin, β-amyrone, camphene, dipterocarpol, germanicol, hydroxy dammarenone , (8*R*)-3-oxo-hydroxy-polypoda-13*E*,17*E*,21-triene, lupeol, malabaricatriene, masticadienonic acid, moronic acid, β-myrcene, 1,4-poly-β-myrcene, nor-β-amyrone, 28-norolean-17-en-3-one, oleanonic acid, oleanonic aldehyde, tirucallol)				gum mastic from *Pistacia lentiscus* Linné, 1758	Figs 23–25; James 1887, pp. 62–63; Behrens 1892, p. 72; Marner *et al.* 1991; Berg *et al.* 1998; Daferera *et al.* 2002; Scalarone *et al.* 2005

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TABLE 8. (Continued)

Name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Preservative	Filler	Remarks	References
caoutchouc cement (Miller's)	ethanol or possibly partly volatile components of Venetian turpentine (see Tab. 5), chloroform	caoutchouc, filtered shellac (button lac), possibly compounds of Venetian turpentine				finally sealed with asphaltum; Rousselet unsure about Venetian turpentine	Figs 10, 11, 23–26; Rousselet 1895, p. 12
Corseal	butyl acetate, chloroform	polymerized linseed oil, any hard plastic waste, polystyrene Thermocol					Figs 12, 13; Sabir 1996
Glyceel, Zut (= Thorne ringing compound)	butyl acetate, industrial methylated spirit (85–90% ethanol, 0–5% methanol), sulfur-free toluene	cellulose nitrate, polymerized linseed oil (Thorne 1935: ADM-100)				Thorne 1935: powdered pigments for colors may be used	Figs 12, 13; Thorne 1935, p. 98; Esser 1973; Hooper 1986a, pp. 77–78; Bates 1997; Brown 1997
Glyptal	5.6% petroleum ether, 0.2% Stoddard solvent (white spirit = aliphatic and alicyclic C7–C12 hydrocarbons), 34.5% xylene	34.7% alkyd polyester resin Glyptal (originally made from glycerol and phthalic acid)			16.9% hydrated magnesium silicate (talc)	color: 8.2% iron oxide; enamel paint for insulating electrical applications and waterproofing car motor parts	Figs 13, 14; 1201Red Enamel Glyptal[1]; Travis 1968; Jordão *et al.* 1996; Deligny & Tuck 2000, p. 32
goldsize (= gold size)	oil of turpentine (not in all recipes)	partially polymerized and oxidized linseed oil			white lead (= basic lead carbonate, $2PbCO_3 \cdot Pb(OH)_2$), red lead (Pb_3O_4), umber (iron oxides, manganese oxide), yellow ochre ($FeO(OH) \cdot nH_2O$)	other recipe: also with asphaltum or with yellow lead oxide (PbO)	James 1887, p. 63; Behrens 1892, p. 71; Gray 1954, pp. 22, 651–654; Beck 1963, p. 23
gutta-percha cement	turpentine	compounds of shellac (see marine glue)				gutta-percha	Figs 12, 26; Gray 1954, p. 656
liquid electrical tape (black)	1993: methylene chloride (= dichloromethane), methyl ethyl ketone, toluene 2008: acetone, methyl ethyl ketone, xylene 2013: acetone, ethylbenzene, methyl ethyl ketone, toluene, xylene	copolymer of poly(vinyl chloride-co-vinyl acetate) with poly(2-propenoic acid, 2-methyl, 2-methylpropyl ester-co-ethenylbenzene-co-2-ethylhexyl 2-propenoate) (= Pliolite AC4)	1993: dioctyl phthalate or castor oil 2008, 2013: diethylene glycol dibenzoate			1993, 2013: epoxy stabilizer: 3,4-epoxy cyclohexyl methyl-(3,4-epoxy) cyclohexane carboxylate (= ERL 4221); dispersant: talc; color: carbon black 2008: Lenplas Eso-1; dispersant: talc; colorant	Figs 12–14; Paynes Marine 29.II.2008[4]; Star brite 30.XII.2013[5]; Dornau *et al.* 1993

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TABLE 8. (Continued)

Name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Preservative	Filler	Remarks	References
marine glue (old formula from 1880)	petroleum ether	compounds of shellac (aleuritic acid, butolic acid, jalaric acid, laccijalaric acid, laccishelolic acid, laksholic acid, shellolic acid)				rubber or gutta-percha; butolic acid = 6-hydroxy tetradecanoic acid; shellac from Hemiptera *Kerria lacca* (Kerr, 1782) and related species	Fig. 26; Gray 1954, pp. 22, 651, 655; Mills & White 1999, pp. 115–118; Colombini *et al.* 2003; Buch *et al.* 2009; Sutherland & Río 2014
Murrayite	benzene	?				invented by C. Hay Murray	Wagstaffe & Fidler 1955, p. 179
nail varnish	butyl acetate, ethyl acetate, isopropyl alcohol, toluene	cellulose nitrate, toluenesulphonamide resin	dibutyl phthalate, camphor, castor oil				Figs 12–14; Travis 1968; Esser 1973; Wells 1978; Hooper 1986a; Orajay 2005a, 2005b; Allington & Sherlock 2007a, 2007b; Horie 2011, p. 214
NOA 61		50-65% of a mercapto-ester, 30-55% triallyl isocyanurate				NOA 61 = Norland Optical Adhesive 61; cured by UV light	Norland Products[2]; Jersabek *et al.* 2010
sealing wax varnish	ethanol, partly volatile components of Venetian turpentine (see Tab. 5) and gum colophony (caryophyllene, *p*-cymene, longifolene, α-terpineol)	compounds of shellac (see marine glue), Venetian turpentine (see Tab. 5), and gum colophony (abietic acid, dehydroabietic acid, isopimaric acid, neoabietic acid, palustric acid, pimaric acid, sandaracopimaric acid, norchrysantemic acid)				pigment; early formulae: also beeswax; colophony (= rosin, Greek pitch) from various species of *Pinus*	Figs 10, 11, 21, 26; Gray 1954, pp. 651–652; Mills & White 1999, p. 100; Scalarone *et al.* 2002
shellac cement	ethanol	compounds of shellac (see marine glue)	Behrens 1892: castor oil			aniline blue, aniline green, or gamboge for color	Fig. 26; James 1887, pp. 63–64; Behrens 1892, p. 72; Gray 1954, p. 654
spirit lacquer	ethanol, partly volatile components of gum sandarac and Venetian turpentine (see Tab. 5)	compounds of gum sandarac and Venetian turpentine (see Tab. 5)	camphor			lamp black for color	Figs 7, 10, 11, 13; Behrens 1892, p. 72

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TABLE 8. (Continued)

Name	Solvent	Potential pre-polymer(s) or polymer	Plasticizer	Preservative	Filler	Remarks	References
silicone rubber	DowCorning®: 0.1–1% methanol; petroleum ether	poly(dimethyl siloxane) like 1–5% methyl trimethoxysilane and 0.1–1% octamethyl cyclotetrasiloxane (see DowCorning®)			calcium carbonate ($CaCO_3$), carbon black	catalysts: organic complexes of metal ions; adhesion promoter: organosilanes like 10–20% hexamethyldisilazane (DowCorning®); pigments; water scavenger	Fig. 12; Dow Corning[3]; Caveness, 1969; Petrie 2007, p. 447; Jersabek *et al.* 2010
white lacquer	benzene, terpentineol, partly volatile components of gum sandarac and Venetian turpentine (see Tab. 5) and gum mastic (see caoutchouc cement)	compounds of gum sandarac and Venetian turpentine (see Tab. 5), gum mastic (see caoutchouc cement), and gum dammar (dammaradienone, dammaradienol, dammarenolic acid, hydroxy dammarenone, nor-α-amyrone, oleanonic acid, oleanonic aldehyde, ursonic acid, ursonic aldehyde)			permanent white (= blanc fixe, barium sulfate, $BaSO_4$)	gum dammar from various species of Dipterocarpaceae, possibly of the genera *Hopea* or *Shorea*	Figs 7, 8, 10, 11, 22–25; Behrens 1892, p. 73; Mills & White 1999, p. 107; Scalarone *et al.* 2005
white zinc lacquer	benzene; (James 1887: nut or poppy seed oil)	compounds of gum dammar (see before)			white zinc (= zinc oxide, ZnO)		Fig. 22; James 1887, p. 64–65; Behrens 1892, p. 73

[1] 1201Red Enamel Glyptal, product information: Available from: http://www.glyptal.com/1201tech001.pdf; material safety data sheet from 28.V.1996: http://siri.org/msds/f2/bsr/bsryg.html (accessed 16 June 2015).

[2] Norland Products: Available from: http://www.norlandprod.com/adhesives/NOA%2061.html (accessed 27 January 2016).

[3] Dow Corning® 3140 RTV Coating: Available from: http://www.dowcorning.com/applications/search/default.aspx?R=109EN (accessed 27 January 2016).

[4] Paynes Marine 29.II.2008: Available from: http://paynesmarine.com/documents/StarbriteLiquidElectricalTape.pdf (accessed 6 April 2016).

[5] Star brite 30.XII.2013: Available from: http://www.starbrite.com/component/com_advlisting/fileId,3099/view,download/ (accessed 6 April 2016).

TABLE 9. Advantages and disadvantages of selected coverslip seals.

Name	Advantages	Disadvantages	References
Araldite		- harmful components (hood!), - curing may take several days or require heat treatment, - adhesion to glass deteriorates at about 70% relative humidity and by loss of plasticizer, - very hard, removable with scalpel only after swelling by solvent	Adam & Czihak 1964; Boparai & Chhabra 1968; Horie 2011, pp. 290, 296
asphaltum		- cracks during drying except if plasticizer is included, - medium permeable for water, - lasts only a few years	Spence 1940c; Travis 1968; McLaughlin 2012, p. 179
neutral Canada balsam	- lasts > 150 years, - diffusion barrier against oxygen (but see text), - easily removable with scalpel	- harmful components (hood!), - balsam may react with phenol and chloral hydrate of gum-chloral media causing blackening of media	Bender 1967; Stroyan in Eastop 1985, p. 269; Brown 1997; Allington & Sherlock 2007a, 2007b
Corseal	- "excellent"	- harmful components (hood!), - ingredients of plastic components unknown and variable, - must be home-made	Sabir 1996; Hooper *et al.* 2005
Glyceel, Zut	- oxidation, excessive dehydration, and crystallization of mounting media delayed, - high plasticity for years, - lasts > 15 years, - easily removable with scalpel	- harmful components (hood!), - not commercially available anymore, must be home-made, - not always permanent	Beer 1954, p. 1110; Esser 1973; Hooper 1986a, 1986b; Bates 1997; Brown 1997; Hooper *et al.* 2005; Neuhaus pers. obs.
Glyptal	- readily available, - lasts > 13 years, - tends "to be flexible and chemically inert", - easily removable with scalpel	- harmful components (hood!)	Travis 1968; Brown 1997; Faraji & Bakker 2008; Walter & Krantz 2009
goldsize	- attaches well to glass, - "remains tacky for a long time", - lasts for many years	- drying time several weeks, - must be sealed with varnish like shellac and lamp black	James 1887, p. 63; Gray 1954, pp. 651–652; Beck 1963
Murrayite	- "impervious to both alcohol and water", - ring balsam first with brown cement and then with Murrayite	- supposed to cause fine granulation of gum-chloral medium, - absorbs glycerol from mounting medium, - often crazes and flakes if slides are stored in a too warm place	Lambers 1950, p. 57; Wagstaffe & Fidler 1955, p. 179; Eastop & Emden 1972; Taylor 2005
nail varnish	- readily available, - easily removable with scalpel	- harmful components (hood!), - ingredients unknown and variable depending on manufacturer, - shrinkage, cracks because of migration of plasticizer out of the varnish, - lacks plasticity, - lasts few years	Travis 1968; Esser 1973; Wells 1978; Hooper 1986a; Hooper *et al.* 2005; Orajay 2005a, 2005b; Allington & Sherlock 2007a, 2007b; Horie 2011, p. 214
silicone rubber	- readily available	- ingredients unknown and variable depending on manufacturer, - silicone releases acetic acid during curing, effect on specimen and medium unknown, - only partly removable with scalpel, special cleaning fluid necessary for re-sealing a mount on the same slide, - limited shelf-live of opened container (moisture starts curing!)	Caveness 1969; Petrie 2007, p. 447

3.7.4 Liquid mounting media (formaldehyde, lactophenol, glycerol, Zeiss W15)

Rousselet (1893, 1895), Myers (1936), and Gray (1954, pp. 21–41) described in detail how cells for fluid-mounted specimens were made. Moore (1979) reported about the restoration of fluid-mounted animal and plant tissue (Tab. 14; see also chapter 3.10.5 Problems with liquid mounting media—re-hydration of specimens). Formulae for liquid media were listed by Gray (1954, pp. 176–181). Such media with a high vapor pressure except formaldehyde, glycerol, and lactophenol are not treated in this article any further. In addition, liquid media used for minerals or cross-sections of film (Loveland & Centifanto 1986), obviously non-permanent media, and most media specifically designed for fluorescence microscope studies are excluded here.

Formaldehyde. Liquid media with a high vapor pressure like formaldehyde (Tab. 10) have been used especially in the 19th and first half of the 20th century for mounting specimens like rotifers (Fig. 15A, B; Rousselet 1893, 1895; Russel 1951; Jersabek 2005). The formaldehyde may be neutralized by some sodium bicarbonate, calcium carbonate, or pyridine; 3–5% glycerol may "increase transparency and minimize evaporation" (Spence 1940b; Morrison 1942). However, Gray (1954, p. 23) and Sanderson (1994, p. 153) recommended not to buffer formaldehyde, because the salts of the buffer might precipitate at some stage. Knudsen (1966, p. 275) suggested 30% glycerol, 3% formaldehyde, and 67% water. All these media bear the inherent risk that slides will deteriorate quickly because of the evaporation of the aqueous part of the medium and crystallization of formaldehyde as is known for various rotifer collections (Fig. 15A, B; Russel 1950, 1951; Jersabeck 2005; Jersabek *et al.* 2010; Schmid et a. 2016; Neuhaus pers. obs.). However, individual slides may keep the liquid medium for at least 120 years, whereas the majority of slides will not (Fig. 15A, B).

Lactophenol. Lactophenol has been advocated as mounting medium repeatedly (e.g., Linder 1929; Franklin & Goodey 1949; Evans *et al.* 1961; Esser 1974; Huys & Boxshall 1991, p. 451), but this medium belongs to the less recommendable liquid mounting media, because both its components lactic acid and phenol possess quite a high vapor pressure (Tabs 10, 13); irrespective of the latter fact, nematode mounts in lactophenol survive for at least 16 years (Esser 1974). Addition of cotton blue, iodine, or Unna's polychrome blue to the lactophenol enhances the contrast of cuticular structures (Linder 1929; Franklin & Goodey 1949; Evans *et al.* 1961). Lactophenol has also been applied together with a gum to give a solidifying mounting medium, but this shows serious signs of deterioration like formation of crystals, darkening of medium, and cracking (Fig. 16A–C).

Glycerol (= glycerin, glycerine). Surprisingly, some liquid mounting media possess quite a low vapor pressure like glycerol (Tabs 10, 13). Glycerol was introduced as a mounting medium in 1849 by Warington (Tab. 1; Bracegirdle 1978, p. 93). Specimen have been mounted in glycerol without (Figs 4A, 5C) or with additional support of the coverslip (Fig. 5B). Support may be temporary, e.g., by thin glass fibers removed after fixation of the coverslip with the synthetic resin NOA 61 (Jersabek *et al.* 2010) or permanent by the double-coverslip technique using Caedax, Canada balsam, Clarite, or Euparal, or by Elmer's Washable Clear School Glue™, Colorations™ Washable Clear Glue, paraffin, wax, or a red rubber ring cemented with goldsize (Ward 1953; Zander 2014; see also chapters 3.8.4 Canada balsam and 3.8.11 Paraffin). For the preparation of a glycerol-paraffin mount see Table 13 Technique E (Hooper 1970, p. 50, 1986a, pp. 76–77; Hooper *et al.* 2005, pp. 70–71). Mounting of rotifers in glycerol on a depression slide was described by Myers (1936). It is important to keep the hygroscopic glycerol anhydrous by storing it in an oven at about 40°C or in a desiccator in order to prevent potential growth of bacteria and fungi (Newell 1947; Neuhaus pers. obs.). Therefore, we are a bit suspicious of a glycerol mount sealed with the hygroscopic Elmer's Washable Clear School Glue™ or Colorations™ Washable Clear Glue (Zander 2014).

Glycerol offers the advantage of "clearing" a specimen, because it reveals a similar refractive index as proteins (Tab. 6; Myers 1936; Maybury *et al.* 1991; Morse 1992, p. 3). Also, glycerol does not change its optical properties over time, does not form cavities and crystals, does not blacken, and has a low vapor pressure of 0.1 pascal at 40°C (comp. with other substances in Tab. 10). Glycerol-paraffin slides are supposed to last "indefinitely" (Myers 1936; Hooper 1986a, p. 68). However, a specimen may move and lose its dorso-ventral orientation in glycerol because of its liquid nature, but this is more a problem during mounting when the paraffin is still liquid and offers some resistance to manipulation of the upper coverslip. Movement of the specimen is not very likely once the specimen is sufficiently flattened and the slide is stored horizontally. Also, the glycerol may evaporate over time and eventually dry out if the coverslip detaches locally from the paraffin and the slide seal breaks (Tab. 6). It seems that glycerol mounts surrounded by paraffin and sealed with Glyceel last longer than glycerol mounts with Glyceel alone (Hooper 1986b, p. 317). Rotifers mounted in glycerol or glycerol-jelly may survive for considerably longer

than 35 years, but no information is available about the kind of seal used for these mounts (Jersabek 2005). Regular control of slides about every five years is recommended for this kind of slide mount and generally, in a longer time interval, for all kinds of slide mounts (see chapter 3.2 Storage; Tab. 6). Glycerol seems to develop acrolein by oxidation over time, which results in a notable absorption peak between 250–310 nm wavelength (Loveland & Centifanto 1986, p. 219, fig. 3). It is unknown whether the formation of acrolein impacts the mount in any way.

In summary, the most safe glycerol mounts seem to be the double-coverslip technique with Canada balsam or Euparal and the glycerol-paraffin technique with a subsequent ringing with Glyceel. Such a properly sealed mount remains stable and fulfills as almost the only mounting medium the criterion of reversibility of mounting at any time, but has to be checked for leakage regularly (Tab. 6). In the senior author's opinion, the former advantages outbalances by far the latter disadvantage, and consequently, the double-coverslip glycerol mount with Canada balsam or Euparal and the glycerol-paraffin mount with a Glyceel seal are recommended as one of the few mounts for museum collections.

Zeiss W15. The liquid medium Zeiss W15 results from a chemical reaction of glycerol with phthalic anhydride and was developed first in the 1950ies (for details see chapter 3.7.23 Zeiss W15, L15, and L25). The medium is still used nowadays, mainly by copepodologists.

3.7.5 Caedax, Caedax A, Caedax 547

Information about Caedax is somewhat confusing, because three different names with at least four different refractive indices for the solid medium are mentioned in the literature (Tab. 5). Lillie *et al.* (1953, p. 69, tab. 1A) referred to a Caedax A from 1951 with a refractive index of the dry resin of $nD^{20°C}$ = 1.6724 in their table but mention a calculated index of nD = 1.6673 in the text; anilin dyes seemed to last in this medium, whereas Gomori-Burtner methenamine silver staining and Prussian blue did not last. Engbert (1957) claimed that Caedax 547 was introduced about 1955 at the latest revealing a refractive index of nD = 1.63. A previous version of Caedax, seemingly introduced in the late 1930s, was supposed to possess a much lower index according to this author. This version may be identical with a medium with an index of $nD^{20°C}$ = 1.558 mentioned by Deutsch (1962) or a medium with an index of $nD^{20°C}$ = 1.5730 (Loveland & Centifanto 1986, pp. 187–188). Diederichs (1932–1933) mentioned Caedax in his article already, so a medium with this name must have already existed in the early 1930s (Tab. 1). The production of Caedax by Merck (originally by Hollborn & Söhne, see Diederichs 1932–1933) seems to have ceased about 1975, because polychlorinated biphenyls used as plasticizers in the polymer were not allowed in open systems anymore (Krauter & Rüdt 1980; Woessner 2005).

Caedax is based on the resin AW2 made by BASF and supposed to be a copolymer poly(cyclohexanone-co-methylcyclohexanone) containing a polychlorinated biphenyl as a plasticizer (Tab. 5; Rie & Shedrinsky 1989; Mills & White 1999, pp. 137–138). The medium was composed in the late 1930s of 13 parts resin AW2, 13 parts of the chlorinated biphenyl Clophen, and 5.3 parts xylene (Kern *et al.* 1946). Resin AW2 was also used for picture varnishes. In 1967, BASF replaced resin AW2 with Ketone Resin N, which seemed to consist of poly(cyclohexanone) only (Rie & Shedrinsky 1989; Mills & White 1999, p. 138). Ketone resin N used as a picture varnish has been reported to yellow over time and to become less soluble (Witte 1983).

The cyclohexanone formaldehyde polymer AW2 is quite brittle and "more prone to autoxidation" than poly(vinyl acetates) and polymethacrylates resulting in a change in solubility (Rie & Shedrinsky 1989). These authors also discussed both the principal pathways of the synthesis of the resin and of its degradation. Kern *et al.* (1946) stated that 10 year-old slides mounted with Caedax were in a perfect condition in 1946. Caedax may be mixed successfully with water-free phenol; crystals may occur, which disappear after heating (Stosch 1952). Small spherical crystals have been found in some slides of the Aphidina collection at the Museum für Naturkunde Berlin (Fig. 15D).

3.7.6 Canada balsam

The first resin mounts go back to 1795 using Venetian turpentine, and mounts in Canada balsam were introduced around 1832 (Tab. 1; Bracegirdle 1978, p. 88). The differences in the refractive indices of Canada balsam at the

same temperature (Tab. 4) may originate from a variable quality of the resin from different manufacturers. Mixtures of resin from *Abies balsamea* (Linné) with resin from other tree species seem to have been sold under the name "Canada balsam" (Spence 1939).

The diterpenoids abietic, levopimaric, neoabietic, and palustric acid in Canada balsam (Fig. 6; Tab. 5) differ from each other mainly in their conjugated double bonds and form an equilibrium, which favors abietic acid after heating (Mills & White 1977, p. 14). Therefore, it can be assumed that the composition of the balsam will change during manufacture of the final product if heating is involved in this process (but see below). Dehydroabietic acid seems to form from the acids mentioned before by oxidative processes and may dominate in older mounts of Canada balsam together with its autoxidized compound 7-oxodehydroabietic acid (Mills & White 1977, p. 14). Abienol and β-phellandrene (Fig. 6; Tab. 5) are supposed to contribute most to the polymerization of Canada balsam (Mills & White 1999, p. 102), but β-phellandrene is actually an ethereal oil. Less volatile components are suggested to act as natural plasticizers (Brunner & Blueford 1986). For some more volatile components, the vapor pressure is found in Table 10. Since the 19th century, the more volatile components seem to be evaporated by heating during manufacture of the balsam, and the latter dissolved in benzene, chloroform, or xylene (James 1887, p. 74; Gray 1954, p. 639). For wholemounts, Gray (1954, pp. 56–57) favored "natural" over "dried" Canada balsam, the latter being meant for mounting sections.

Raw Canada balsam is composed of many unsaturated compounds (Fig. 6; Tab. 5; Bender 1967), which are assumed to bleach stains like Prussian blue "by converting it to the greenish white ferrous ferrocyanide" but "preserve cobalt sulfide well" (Lillie *et al.* 1953, p. 58). The acids contained in Canada balsam (Tab. 5) cause cationic stains of histological sections to fade and carbonate structures to dissolve (Southgate 1923; Kirkpatrick & Lendrum 1939; Spence 1939; Davies 1940; Romeis 1948; Adam & Czihak 1964; Böck 1989; Brown 1997; Kiernan 2015, p. 67). In the 1930s, the neutral Canada balsam was not "neutral" at all revealing a pH from 4.1–8.7 (Kirkpatrick & Lendrum 1939; Davies 1940) despite neutralization with potassium carbonate or alkali earth carbonates (Southgate 1923; Lillie *et al.* 1953; Adam & Czihak 1964; Böck 1989). It remains open whether or not current Canada balsam from different manufacturers reveals a neutral pH. Even neutralized mounting media based on natural resins are supposed to develop acidity over time again, probably because of ongoing degradation processes in the resin (Krauter & Rüdt 1980, p. 264).

For Kinorhyncha and helminths, dehydration via ethanol and xylene and mounting in Canada balsam dissolved in xylene has been found to harden specimens too much, such that they break into pieces during mounting (Pritchard & Kruse 1982, p. 122; Berland 1984; Neuhaus 2013, p. 278). Xylene seems to harden tissues and especially gonads with yolk more than almost all other transition media (Gatenby & Beams 1950, p. 70; Wagstaffe & Fidler 1955, p. 173; Sanderson 1994, p. 42). This problem is overcome for Copepoda and a range of other meiofauna organisms by soaking a specimen in phenol and mounting the specimen in phenol balsam (Gatenby & Beams 1950, p. 90; Thatcher 1987; Brown 1997, p. 8). However, inclusion of phenol may lead to excessive darkening of the mounting medium (see discussion in chapter 3.7.25 Discoloration). Possibly, substituting Cellosolve™ or Histoclear for xylene may avoid or diminish excessive hardening of a specimen as has been suggested for Euparal (comp. chapter 3.7.12 Euparal). Also, *n*-butanol, 1,4-dioxane (toxic), and terpineol may serve this purpose (Gatenby & Beams 1950, pp. 89–91). In addition, dehydration with isopropyl alcohol instead of ethanol may also lead to less hardened specimens (Gatenby & Beams 1950, p. 70).

Delicate morphological characters are masked by Canada balsam in kinorhynchs, diatoms, helminths, and mites (Fleming 1943; Barr 1973; Heikinheimo 1988; Neuhaus pers. obs.), but the medium seems to be suitable for certain insects (Clarke 1941; Essig 1948; Palma 1978). Possibly, the masking problem may also be overcome by emulating different contrast methods with special software (comp. chapter 3.12.1 Microscope equipment).

Canada balsam yields several disadvantages, which may discourage its use in comparison with other media (see discussion above; Tab. 6). However, little is known about the drawbacks of most other mounting media, because most articles enthusiastically report the benefits of newly developed media but rarely summarize anecdotal information about the longevity of a given mounting medium (Upton 1993; Brown 1997). Systematic long-time studies on these media are missing entirely. Mounting media based on Canada balsam seem to have been produced with variable quality in the past, which certainly accounts for problems with stains (see next paragraph). And because Canada balsam has been in use for more than 180 years, information about this medium is considerably more comprehensive than on any other medium except Euparal and the gum-chloral media (Upton 1993).

TABLE 10. Vapor pressure of selected volatile components of mounting media and coverslip seals as well as of an immersion oil taken from GESTIS Substance Database (= Information system about hazardous substances; Available from: http://gestis-en.itrust.de/nxt/gateway.dll/gestis_en/000000.xml?f=templates$fn=default.htm$3.0 accessed 14 January 2016 and 04 April 2017) except if noted otherwise.

Chemical name	**Alternative name**	**Vapor pressure** [hPa, at 20°C]	**Aggregation state** [at room temperature]	**Notes**
acetic acid	glacial acetic acid	15.8	liquid	melts at 17°C; Fig 13
acetophenone		0.4	liquid	melts at 20°C; Fig. 9
anethole		1.35 [65°C]	liquid	melts at 21.3°C; Fig. 23
aniline oil	phenylamin	0.681	liquid	
anise oil	anisole, methyl phenyl ether	3.2	liquid	Fig. 13
benzaldehyde		1.26	liquid	Fig. 9
benzene	benzol	100	liquid	melts at 5.5°C
benzoic acid		0.001	solid	Fig. 9
benzyl benzoate		< 0.1	liquid, solid	melts at 21°C; Fig. 13
butyl acetate		10.7	liquid	Fig. 13
tert-butyl alcohol	2-methyl-2propanol	41.2	liquid	Fig. 13
camphene		ca. 21.5	solid	melts at 45–48°C; Fig. 9
camphor [2]		0.24	solid	sublimes; Fig. 8
carbon tetrachloride		119.4	liquid	
Δ^3-carene		2.7	liquid	Fig. 6
castor oil	ricinus oil	< 0.001	liquid	
Cellosolve™	ethylene glycol monoethyl ether, 2-ethoxyethanol	5.06	liquid	
chloral hydrate [1]	2,2,2-trichlorethane-1,1-diol	13	solid	melts at 52°C; sublimes; Fig. 13
chloroform		209	liquid	Fig. 13
p-cymene	*p*-cymol, dolcymene	1.45	liquid	Fig. 21
dibutyl phthalate [1]		0.026	liquid	Fig. 14
dieethylene glycol		0.008	liquid	

……*continued on the next page*

TABLE 10. (Continued)

Chemical name	Alternative name	Vapor pressure [hPa, at 20°C]	Aggregation state [at room temperature]	Notes
diethyl ether		586	liquid	
diethyl phthalate		0.00144 [30°C]	liquid	Fig. 14
2,6-di-*tert*-butyl-*p*-cresol	butylhydroxy toluol	0.02	solid	Fig. 13
dioctyl phthalate		0.01	liquid	Fig. 14
ethanol		58	liquid	Fig. 13
ethyl acetate		98.4	liquid	
eucalyptol	1,8-cineole	1.22	liquid	melts at 1.5°C; Fig. 8
formaldehyde		2	gaseous	
glycerol	glycerin, 1,2,3-propanetriol	0.00121 [40°C]	liquid	melts at 18°C; Fig. 13
n-heptane		47.4	liquid	
n-hexane		162	liquid	
hydrocinnamyl alcohol	3-phenyl-1-propanol	0.5	liquid	
iodine[2]		0.4 [25°C]	solid	
isoamyl acetate	3-methylbutyl acetate	5.3	liquid	Fig. 13
isopropyl alcohol	2-propanol, isopropanol	42.6	liquid	
lactic acid		0.1 [25°C]	liquid, solid	melts at 53°C; Fig. 13
limonene	dipentene, cajeputene	2.04	liquid	Fig. 6
linalool		0.1	liquid	Fig. 23
(R)-*p*-mentha-1,8-diene	terpinolene	0.7	liquid	
methyl benzoate	Niobe oil	0.25	liquid	Fig. 13
methyl salicylate	wintergreen oil	0.13	liquid	Fig. 13
methylene chloride	dichloromethane	470	liquid	boils at 40°C

……*continued on the next page*

TABLE 10. (Continued)

Chemical name	**Alternative name**	**Vapor pressure** [hPa, at 20°C]	**Aggregation state** [at room temperature]	**Notes**
myrcene		2.78 [25°C]	liquid	Figs 6, 23
oil of turpentine	turpentine oil, wood turpentine	5	liquid	not Venetian turpentine
paraldehyde	2,4,6-trimethyl-1,3,5-trioxan	10.3	liquid	melts at 12°C; Fig. 8
petroleum ether	ligroine	400	liquid	
phenyl salicylate [3]	salol	<0.133	liquid	melts at 41°C; Fig. 8
phenol [2]	carbolic acid	0.47 [25°C]	solid	melts at 41°C; liquid if water is present; Fig. 13
α-pinene		5 [25°C]	liquid	Fig. 6
poly(ethylene glycol) 200–600		< 0.1	liquid	melts at 4–8°C
resorcinol [2]		2.66 x 10^{-4} [25°C]	solid	Fig. 9
styrene	cinnamol, styrol	7.14	liquid	Fig. 9
terpineol	synthetic oil of lilac	0.24	liquid	Fig. 11
tetrahydrofuran		173	liquid	Fig. 13
thymol	2-isopropyl-5-methylphenol	2.5 [50°C]	solid	melts at 49–51°C
toluene	toluol	29.1	liquid	Fig. 13
water		23	liquid	
xylene (*o*-, *m*-, *p*-isomers)	xylol	8–10	liquid	Fig. 13
Zeiss Immersol™ 518 F [4]		< 0.1	liquid	immersion oil

[1] Data from Wikipedia: Available from: https://www.wikipedia.de/ (accessed 14 January 2016).
[2] Data from NIOSH Pocket Guide to Chemical Hazards: Available from: http://www.cdc.gov/niosh/npg/ (accessed 14 January 2016).
[3] Data from Carolina: Available from: http://www.esciencelabs.com/sites/default/files/msds_files/Salol.pdf (accessed 30 January 2017).
[4] Data from Zeiss: Available from: https://www.micro-shop.zeiss.com (accessed 30 March 2016).

TABLE 11. Diffusion and permeability coefficients of polymers for oxygen and water vapor. For permeability dimensions and exponent of ten (here given as negative value) see chapter 3.7.2 Permeability for gases and vapors. Data partly re-calculated for easier comparison. * = also original reference checked cited by Wypych (2012); [a] = at 35°C; [b] = unbleached shellac in ethanol; [c] = bleached and de-waxed shellac in ethanol.

Polymer	**Polymer as ingredient of mounting medium or coverslip seal**	**Diffusion coefficient** [$cm^2\ s^{-1}\ x\ 10^6$]		**Permeability coefficient** [$cm^3\ cm\ cm^{-2}\ s^{-1}\ Pa^{-1}\ x\ 10^{-12}$ at 25°C]		**Reference**
		O_2	**H_2O**	**O_2**	**H_2O**	
carboxy methyl cellulose (CMC), glycerol	CMC?	-	-	-	0.1366	Wypych 2012 *
cellulose hydrate	cellophane	-	-	0.00016	1,890	Pauly 1999
cellulose nitrate (CN)	Glyceel, nail varnish	0.15	0.0262	0.146	472	Pauly 1999; Wypych 2012
methyl cellulose (MC)	C-M Medium	-	-	0.0375	0.755	Comyn 1986, p. 61; Wypych 2012 *
epoxy resins	Araldite, Epon™, Spurr's	-	-	-	4–30	Gerlach *et al.* 2001
gelatin (GEL)	glycerol-gelatin		3–9.6	88.9	3,500	Wypych 2012
hydroxypropyl cellulose (HPMC)	Klucel G®	-	283	-	-	Wypych 2012
poly(dimethyl siloxane)	silicone rubber	-	-	0.695 [a]	-	Pauly 1999
silicones	silicone rubber	-	-	7.6–46	800–3,200	Gerlach *et al.* 2001
poly(ethyl methacrylate) (PEMA)	coverslip seal	0.106	0.102	0.0889	238	Pauly 1999
poly(2-hydroxyethyl methacrylate) (PHEMA)	Technovit® 7100	-	0.59–5.37			Wypych 2012
poly(methyl methacrylate) (PMMA)	Lucite™	0.005	-	0.0115	48–190	Pauly 1999; Gerlach *et al.* 2001; Wypych 2012
polystyrene (PS)	DePeX/DPX, Corseal	-	-	0.19	135	Pauly 1999; Wypych 2012
poly(isoprene) (amorphous; natural rubber)	gutta-percha cement	1.73	-	1.76	-	Pauly 1999
poly(*trans*-isoprene) (vulcanized purified gutta-percha)	gutta-percha cement	0.7	-	0.464	37.9	Pauly 1999
poly(vinyl acetate) (PVAC)	liquid electrical tape, Solvar S.357	0.0562	-	0.0367	-	Pauly 1999; Wypych 2012
poly(vinyl acetate-co-vinyl chloride)	liquid electrical tape, Vinylite	-	-	0.18	5.25	Comyn 1986, pp. 61, 63
poly(vinyl alcohol) (PVOH)	polyvinyl lactophenol, Solvar S.357	-	0.746	0.000665	0.525	Pauly 1999; Wypych 2012
poly(vinyl chloride) (PVC)	liquid electrical tape	0.012	-	0.0034	20.6	Pauly 1999
gum dammar	white lacquer, white zinc lacquer	-	-	-	0.00132–0.00473	Pethe & Joshi 2013
shellac	caoutchouc cement (Miller's), gutta-percha cement, marine glue, sealing wax varnish, shellac cement	-	-	0.0127 [b], 0.0075 [c]	0.0463 [b], 0.0838 [c]	Hagenmaier & Shaw 1991

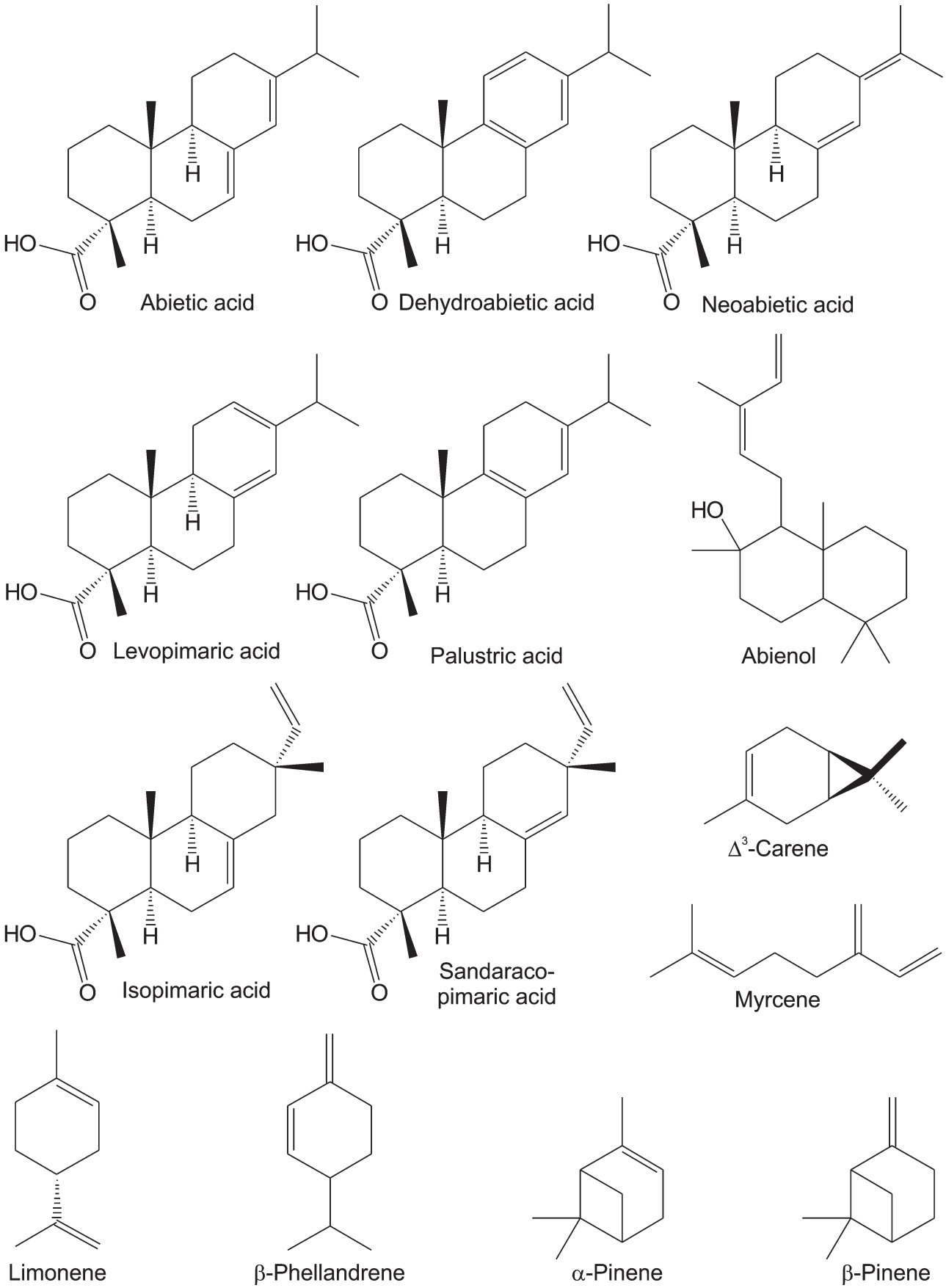


FIGURE 6. Chemical structure of the ingredients of Canada balsam from *Abies balsamea*.

Welsby (1951) bleached Canada balsam experimentally by exposure to sunlight for 110 hours to up to 133 days. The idea of bleaching Canada balsam and other natural resins by exposure to sunlight was already practiced in the 19th century (Edwards 1871). However, this procedure may initiate unwanted chemical processes as has been documented for another natural resin, gum mastic; here, exposure to sunlight for a few days during harvesting starts ongoing autoxidation (Dietemann *et al.* 2001). This is in accordance with findings of oxidized di- and triterpenoids in raw gum dammar, gum mastic, and gum colophony (Scalarone *et al.* 2005).

The biggest advantage of Canada balsam from a curatorial point of view is that it lasts at least 150 years on microscope slides (Brown 1997) and, unlike many other mounting media, is supposed not to deteriorate in a variety of climates (Mound & Pitkin 1972). Nevertheless, Canada balsam yellows and darkens with age, which does not seem to impact bright field microscope studies too much (Welsby 1951; Eastop 1985; Brunner & Blueford 1986; Thatcher 1987; Halliday 1994; Brown 1997), at least not as long as the medium does not become too dark. Darkening seems to start from the periphery (Fig. 15E; Essig 1948) suggesting influence of oxygen diffusion (Loveland & Centifanto 1986, p. 184). Darkening may also be caused by clearing in clove oil (Wagstaffe & Fidler 1955, p. 173). Excessive darkening and finally blackening may result from phenol in the resin, which seems to start from the periphery of the mount (comp. chapter 3.7.25 Discoloration; Brown 1997, fig. 23).

Canada balsam was also applied in a mixture with the plasticizer camphor and with phenol (Spence 1940a). A specimen may be mounted directly from chloroform, 80% ethanol, glacial acetic acid, and other macerating agents. However, un-ringed slide preparations develop crystals, probably because of the evaporation of camphor and phenol, whereas ringed mounts last at least 9 years. Also, many stains will not last (Spence 1940a).

3.7.7 Celodal, Celochloral

Introduced for mounting macroscopic specimens in 1938, Celodal by Bayer, Leverkusen has also been used for mounting specimens on microscope slides, probably mainly in Germany based on the literature found (Tab. 1; Kaudewitz 1951–1952; Krauter 1952–1953; Ant 1957; Hirling 1957–1958; Ossiannilsson 1958). The resin is based on a urea-formaldehyde polymer. The latter is cross-linked by an acid catalyst or heat resulting also in the formation of water molecules (Horie 2011, p. 297). The addition of an acidic catalyst like oxalic acid or trichloroacetic acid will lead to a brittle resin, which does not attach to glass surfaces if the mount is not ringed with a coverslip seal in order to prevent total loss of water. However, a specimen can be mounted directly from ethanol, formaldehyde, isopropyl alcohol, and water without curing with an acid (Kaudewitz 1951–1952; Krauter 1952–1953; Ant 1957; Hirling 1957–1958). Because of its refractive index, Celodal seems to physically clear specimens (Krauter 1952–1953; Ant 1957).

Celodal has been modified to "Celochloral" by adding chloral hydrate, glacial acetic acid, and glucose (Ossiannilsson 1958). However, sometimes rhomboid crystals develop within a few months in the storage bottle. Also, too much water in the medium may lead to "milky cloudiness" and transfer of too much phenol from maceration in chloral hydrate plus phenol to Celodal may result in "milky turbidity" (Ossiannilsson 1958). On a slide at the Museum für Naturkunde Berlin with a specimen of Aphidina mounted in Celochloral 1965 and un-ringed, the peripheral medium turned yellow, whereas the central medium remained clear (Fig. 15F).

Microscope slides mounted with Celodal seem to last at least four years (Ossiannilsson 1958). The high amount of cross-links of the polymer cannot be dissolved again, but has to be destroyed chemically "by concentrated acids or alkalis or molten phenol" (Horie 2011, p. 300) or by "keeping the slides for a few hours in hot water or in hot 10% KOH" (Ossiannilsson 1958).

3.7.8 C-M Medium and CMC / CMCP

There is some confusion about the composition of several media named C-M Medium, CMC-9, CMC-10, CMCP-9, and CMCP-10 (Tab. 5). Clark & Morishita (1950) introduced a new mounting medium "C-M Medium" (= Clark-Morishita Medium) composed of methocellulose (= methyl cellulose, Methocel™; Tab. 12), carbowax 4,000 (= poly(ethylene glycol)), diethylene glycol, ethanol, lactic acid, and water as ingredients (Tab. 1). The same ingredients were listed subsequently by Evans *et al.* (1961), Singer (1967), and Krantz (1978). In carboxy methyl cellulose, the three hydroxyl groups of each β(1→4)-linked D-glucose unit are substituted to a variable degree with

carboxymethyl groups (Fig. 12); the product is generally abbreviated "CMC" (Stelzer & Klug 1980). Zander (1983) created his lactophenol gel by mixing methyl cellulose, ethylene glycol, lactic acid, phenol, and water (Tabs 12, 13) in search of a medium to replace gum-chloral media, which became regulated and difficult to purchase in the USA.

Stehr (1987, p. 16) produced a medium "CMCP-9" in his lab by mixing one of the poly(vinyl alcohols) (!) of the Elvanol® group from DuPont with lactic acid, phenol crystals, and water (Tabs 12, 13); he acknowledged the company Turtox/Cambosco for the recipe. The MSDS of CMC-9, CMC-10, CMCP-9, and CMCP-10 of EMS and Polysciences (Tab. 5) state as ingredients (fully hydrolyzed) poly(vinyl alcohol), lactic acid, phenol, and water. However, Brown (1997, p. 12) listed Clark & Morishita's (1950) recipe under the heading "Carboxy methyl cellulose CMC = Cellofas & Turtox" and on page 8 under the title "3.3.1 Cellofas = Carboxy Methyl Cellulose (CMC) (RI = 1.428)" treated the C-M Medium by Clark & Morishita (1950) and CMC as identical media. This is amazing, because according to the references cited above the two media contain different polymers, viz, either methyl cellulose and poly(ethylene glycol) or fully hydrolyzed poly(vinyl alcohol). It cannot be excluded that CMC(P) contains also methyl cellulose and poly(ethylene glycol), but the percentage proportions of ingredients provided by the MSDS of Polysciences (Tab. 5) do not indicate that another substance is included in the medium in a significant amount. The composition of CMC may have changed over time, because Michelson (1960) and Davis (1964) stated that stained specimens could not be mounted in CMC, whereas Becker & Heard (1965), McHardy (1966), and Mikkelsen (1985) reported that CMC worked well with stains like acid fuchsin, aniline blue, lignin pink, and pronticil dye.

Poly(ethylene glycol) oxidizes at room temperature in the presence of air and light but also in the dark releasing formaldehyde and long-chain aldehydes (Horie 2011, p. 191). CMC seems to dry quicker than CMCP (Mikkelsen 1985). CMC-10 lasts at least one year (Michelson 1960). Specimens of *Zelinakderes* sp. mounted in 1993 in CMCP-10 revealed crystals as early as 1999 already (Fig. 15G, H). Also, these specimens were barely visible in 2015, probably because of the macerating activity of lactophenol included in the mounting medium.

CMC-10 is also used for immobilizing crustacean appendages on a slide and providing a supportive ring for the coverslip, before the mount is prepared with polyvinyl lactophenol (McHardy 1966). Such mounts last at least four years.

3.7.9 Cyanoacrylate

Cyanoacrylates like Bostik Super Glue™ and Technicoll-C have been used to mount semithin sections mounted in a synthetic resin on microscope slides since the 1960s (Molnar & Molnar 1967; Geysen & Loof 1983; Liu *et al.* 2010). Recently, such a glue has also been suggested for mounting type and voucher material for museum collections, viz, Loctite® Super Glue containing ethyl cyanoacrylate; slides last at least one year (Criado-Fornelio *et al.* 2014). Semi-thin sections mounted in cyanoacrylates seem to last at least 15 years (Liu *et al.* 2010). This is contrasted by reports that cyanoacrylate adhesives break down after a short time because of the interaction with the alkaline surface of soda-lime glass (Jackson 1982; Robson 1992, p. 187; Davison 2003, p. 217; Horie 2011, p. 166), in this case the microscope slide. The polymerization of cyanoacrylates seems to be irreversible (Liu *et al.* 2010), but N,N-dimethyl formamide and dimethyl sulfoxide are reported to dissolve the polymer again (Molnar & Molnar 1967). Extended exposition to acetone or light may cause degradation of ethyl cyanoacrylates (Horie 2011, p. 158).

3.7.10 DPX / DePeX

According to the literature, DPX and DePeX contain the polymer distrene 80, a polystyrene (Fig. 12), and the plasticizer dibutyl phthalate replacing tri-*o*-cresyl phosphate of older formulae (Figs 13, 14; Tab. 1; Kirkpatrick & Lendrum 1939, 1941). The two media seem to differ only in the relative proportions of the polymer and the plasticizer (Roe *et al.* 1991). The plasticizer is necessary to avoid collapse of the medium (Kirkpatrick & Lendrum 1939, pp. 592–593; Davies 1940, p. 469) and to provide better adhesion to the glass surfaces (Loveland & Centifanto 1986, p. 184). However, the plasticizer may evaporate over time (for vapor pressure comp. Tab. 10). More recently, the formula concerning the volatile components seems to have changed in order to allow use of the

medium with coverslipping machines. As a consequence of this, slides have to be air-dried and should not be dried in an oven, because the heat will lead to the development of numerous gaseous bubbles in the medium (Göke 2000; R. Sluys pers. com.). Based on IR-spectroscopy, **Fluorolite**, **Hystomount**, **Styrolite**, and **UV-inert** agree with DPX and DePeX in their general chemical composition, a mixture of a polystyrene, dibutyl phthalate, and xylene (Roe *et al.* 1991). Differences in the refractive indices of these mounting media are supposed to be related to the different proportions of the polystyrene and the plasticizer.

Generally, DePeX is considerably better suited for mounting sections rather than total mounts, because the medium loses a considerably amount of volume on drying (Göke 2000). DePeX is recommended also for immunofluorescence microscopy (Göke 2000), because "the fluorescent signal remains high and stable" for more than one year in slides stored at room temperature in the dark as opposed to aqueous mounting media (Espada *et al.* 2005). Kirkpatrick & Lendrum (1941, p. 442) observed formation of quite colorless "thin, needle-like crystals" and "small irregularly curved flat plaques" in DPX if the xylene used for clearing the sections contained remnants of paraffin. Small accumulations of crystals are also found in the mounting medium close to but not on the histological sections of microscope slides at Berlin (Fig. 15I, J).

For diatoms, a mounting medium composed of polystyrene, toluene, and methylene iodide results in a high refractive index $nD^{24°C} = 1.75$ (Czarnecki & Williams 1972). The long-time stability of polystyrenes is questioned because of their light-sensitivity (Horie 2011, p. 181; Wypych 2013, pp. 461–462).

3.7.11 Epoxy resins (Araldite, Epon™, Spurr's resin)

Epoxy resins like Araldite, Epon™, and Spurr's resin are mainly used for embedding biological specimens for transmission electron microscopy. The pre-polymers consist either of diglycidyl ethers of bisphenol A (Araldite: Tabs 5, 8), triglycidyl ethers of glycerol (Epon™), or vinyl cyclohexane dioxide (Spurr's resin); the resin also contains an accelerator, hardener, plasticizer, and in some cases a flexibilizer (Sanderson 1994). Flexibilizers are chemically part of the cross-linked polymer, whereas plasticizers are located between polymer strands and bound by the weaker hydrogen bonds (Sanderson 1994, p. 65). "Slower polymerization at a lower temperature will produce a softer block", which will allow more satisfying staining (Sanderson 1994, p. 65). It should be kept in mind that components of epoxy resins are quite hazardous.

Spurr's low viscosity embedding medium has been used for mounting plant material on a microscope slide (Herr 1982). Bulk preparations of meiofauna has been mounted in a mixture of Epon™ and Araldite or in Epon™ 812 for both ecological and morphological studies; each mount containing hundreds of specimens may be 1–3 mm thick (Rieger & Ruppert 1978). Specimens may be observed both with a stereo microscope and with a compound microscope if the mount is inverted to observe the specimens, which dropped to the bottom of the mount. Although no coverslip is involved, useful information may be obtained even with oil immersion objectives by applying the oil directly to the surface of the mounting medium (Rieger & Ruppert 1978). These authors also suggest cutting out an individual specimen with a small coping saw and either re-mounting the specimen for TEM studies or dissolving the resin (see below; Winborn & Guerrero 1974) and to prepare the specimen as whole mounts either for light microscopy or for SEM studies. Alternatively, specimens may also be sectioned for light microscopy (Smith & Tyler 1984). The Epon™-Araldite resin of the sections on the glass slide is dissolved in methanol saturated with potassium hydroxide and stained with alcian blue, Mayer's mucicarmine, or Heidenhain's iron hematoxylin with or without eosin Y as counterstain (Smith & Tyler 1984). Sections mounted in Epon™-Araldite reveal fewer shrinkage artifacts than sections mounted in paraffin or ester wax media and can be sectioned thinner leading to improved resolution. Also, fixation in a mixture of glutardialedehyde and formaldehyde results in "superior structural preservation" (Smith & Tyler 1984).

"The adhesion of epoxy resins to glass deteriorates sharply above a critical RH (≈70%) due to the absorption of water by the hydroxyl groups in the epoxy and loss of hydrogen bonding to the glass by displacement of water" (Horie 2011, p. 296). A high relative humidity can be expected especially in tropical countries. Adhesion may also be reduced by loss of the plasticizer over the years (Horie 2011, p. 290). Epoxy resins based on aromatic components are more prone to deterioration by light, UV-light, and heat than resins based on aliphatic components (Horie 2011, p. 290). Various silanes may be used as adhesion promoters for glass, but these molecules are quite permeable for water and may undergo hydrolysis if exposed to water for a longer time (Wypych 2013, p. 373).

Once cured, epoxy resins are supposed not to be soluble again, but only to be removable mechanically after swelling by solvents (Horie 2011, p. 296). However, Böck (1989, pp. 150–151) provided two recipes for dissolving epoxy resins either in sodium methylate in methanol and subsequently in acetone or alternatively in sodium hydroxide in water-free ethanol. Epon™-Araldite may be dissolved with methanol saturated with potassium hydroxide (Smith & Tyler 1984). Epon™ 812 may be removed from specimens mounted for transmission electron microscopy with Epox-E-Solv (Winborn & Guerrero 1974). TAAB Resin Resolve softens Araldite, and ethyl acetate finally dissolves it (Russel 1989). Acetic acid, chloroform, dimethyl formamide, methylene chloride, and tetrahydrofuran remove epoxy resin adhesives most effectively (Down 2001, pp. 42, 44). Spurr's resin can be dissolved with potassium or sodium hydroxide in anhydrous methanol; if the methanol contains traces of water, even from humidity, sections may detach from the glass slide (Vermathen 1993). All methods above appear quite drastic and noxious, so they certainly do not qualify for objects of art, which Horie (2011) had in mind. Tests should be made before more general application to microscope slides.

3.7.12 Euparal

Euparal was introduced by Gilson (1906; Tab. 1), but he did not publish the complete formula of the chemical components. Instead, he passed his recipe to commercial companies and made them produce the medium. It seems that for this reason Euparal has been disliked by scientists for a long time (Bracegirdle 1978, p. 91). Possibly, Euparal is also either marketed under the name **Diaphane** in a colorless or green formula (Essig 1948, p. 17; Loveland & Centifanto 1986, p. 193), or Diaphane contains *inter alia* "juniper gum and various natural and synthetic phenols" (Lillie *et al.* 1953, p. 64). Gum sandarac may be identical with gum juniper according to Denham (1923, p. 193).

Raw gum sandarac, the resin component of Euparal (Figs 7, 8; Tab. 5), seems to contain low molecular weight polymers of polycommunic acid "still fairly soluble in rather polar solvents" (Mills & White 1999, pp. 99, 102). Communic acid and related compounds may possess a high potential for cross-linking because of the reactive conjugated double bonds in the side-chain of the molecule (Fig. 7); this would result in the low molecular-weight poylmer polycommunic acid (Mills & White 1977, p. 15). During experimental ageing of gum sandarac, the degree of cross-linking increases over time, but fragmentation also occurs (Scalarone *et al.* 2003a). For the refractive index see Table 4. Euparal comes either as a slightly yellowish or as a greenish medium termed Euparal green or vert. The green color is supposed to enhance contrast in hematoxylin stains (Lillie *et al.* 1950, p. 4, 1953, p. 64; Wagstaffe & Fidler 1955, p. 175; Böck 1989, p. 297). Copper salts (Lillie *et al.* 1953, p. 64), possibly of abietinate (Shepherd 1918, p. 132), seem to be responsible for the green color. The medium has a reported long-time stability of over 50 years (Brown 1997; Brown & Boise 2005, 2006).

Feulgen stain fades by about 30% within two weeks if sections are mounted in Euparal (Dewse & Potter 1975). Small and delicate marine plankton fixed to a glass slide with a thin layer of Euparal may be stained with Delafield's hematoxylin base or fuchsin acid (Veer 1982). Probably, Copepoda, Kinorhyncha, and mosses do not mount well in Euparal, because dehydrated specimens and their organs become brittle and consequently break during mounting (Garner & Horie 1984; Koomen & Vaupel Klein 1995; Neuhaus & Sørensen 2012; Neuhaus 2013, p. 278; Sørensen & Pardos 2008, p. 35). This may possibly be overcome by substituting Cellosolve™ or Histoclear for Euparal Essence, which is used to dilute the Euparal resin (Brown 1997, p. 8; see also additional suggestions in chapter 3.7.6 Canada balsam). Histoclear II (= Histolene) contains 70–90% aliphatic hydrocarbons and 10–30% limonene (National Diagnostics: Available from: https://www.nationaldiagnostics.com/histology/product/histo-clear-ii, accessed 11 February 2016). Other substitutes with similar properties are listed in Wynnchuk (1994). However, tests should be conducted in any case, because problems with fading stains have been documented for Histoclear (Staruk 1985; Buesa & Peshkov 2009). Euparal is documented to last generally more than 50 years (Tab. 6; Brown 1997), but according to a single report the medium seems to "polymerize and craze" at the margin of the coverslip within 7 years (Halliday 1994). Based on reports from the Smithsonian Institution, Euparal from Chroma (Germany) seems to be less satisfying than Euparal from ASCO (Great Britain); however, no specific problems are mentioned (Brown 1997, p. 8). In summary, especially the long-time stability over at least 50 years and mounting from a higher concentration of ethanol suggests Euparal as a recommendable medium for museum collections.

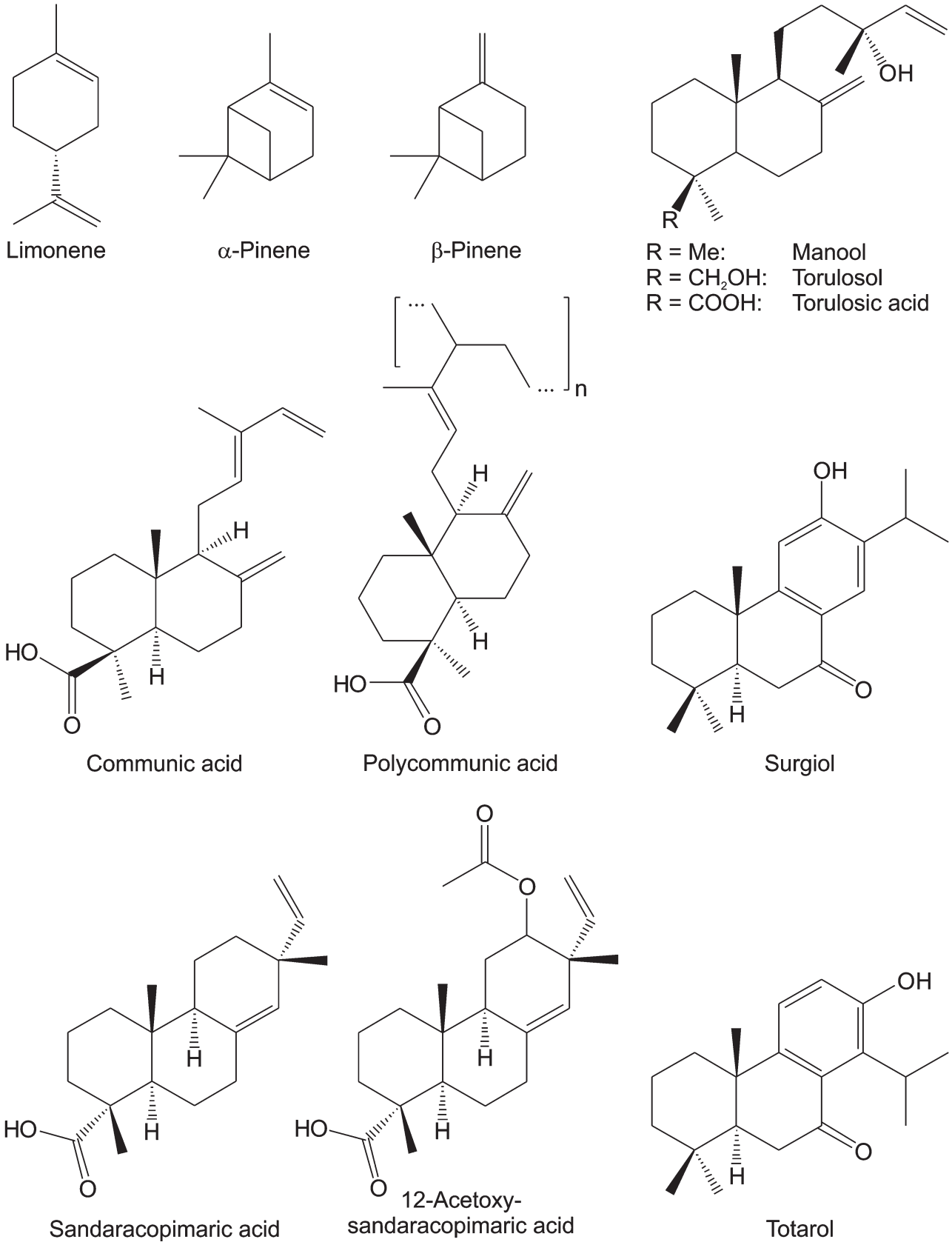


FIGURE 7. Chemical structure of the ingredients of sandarac resin from *Tetraclinis articulata* in Euparal. Me, methyl.

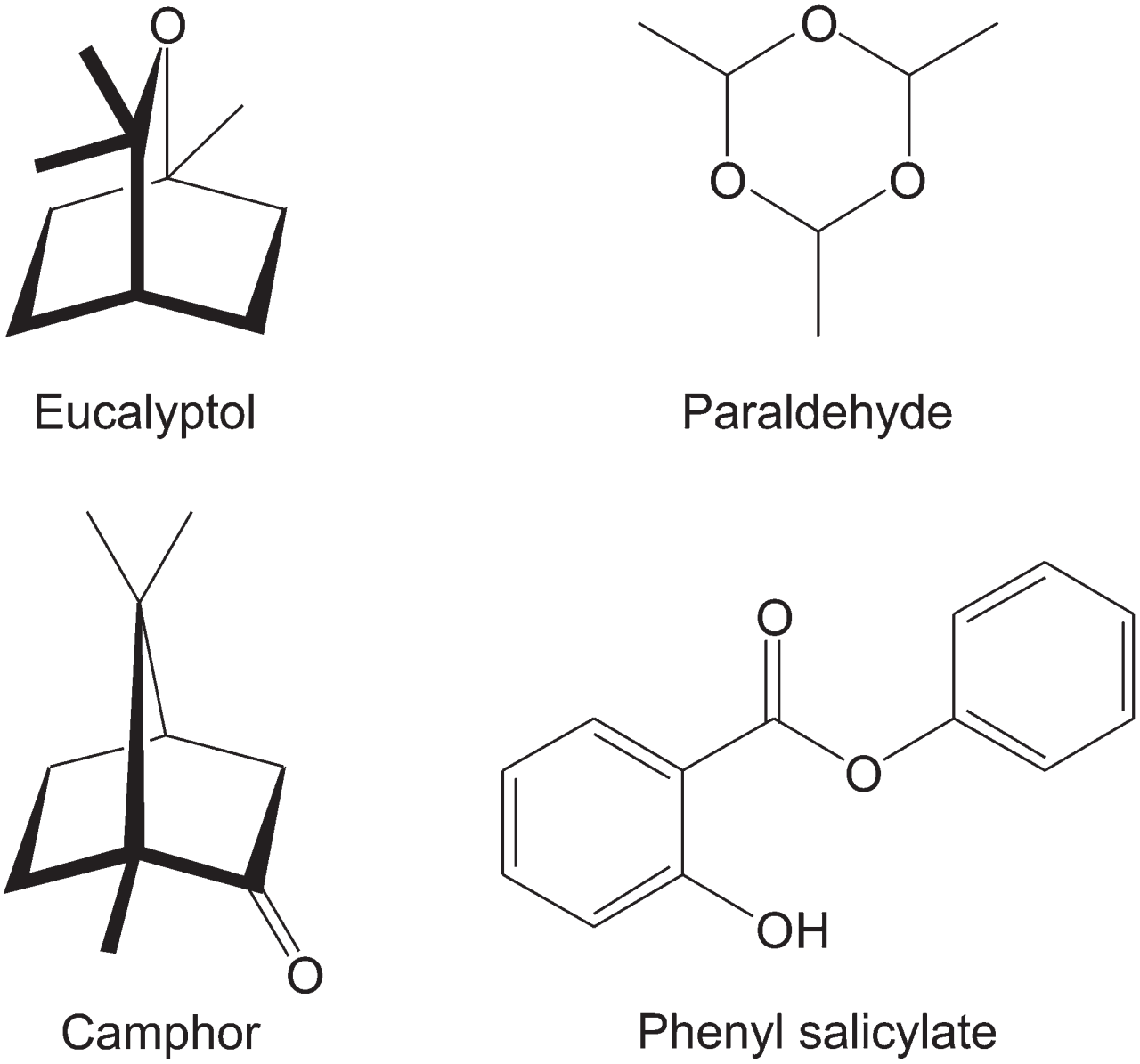


FIGURE 8. Chemical structure of the other potential ingredients of Euparal.

Gum sandarac, camphor, and isobutyl alcohol may be mixed with chloral hydrate (camphloral), phenol (camphenol), and thymol (camthymol) (Denham 1923, pp. 193–194). Camphenol seems to work well with sections stained with hematoxylin and eosin but not with methylene blue and thionin (Kernohan 1928). Probably, these media will suffer from evaporation of camphor and phenol in the long run because of the high vapor pressure of these substances (comp. Tab. 10). Spence (1940a) mentioned a mixture of amyl alcohol, castor oil, and gum sandarac. Mohr & Wehrle (1942) mixed camphor, copper oleate, eucalyptol, paraldehyde, phenyl salicylate, and gum sandarac; dioxan was added or not. If the storage bottle is kept in the dark, the medium will yellow considerably, whereas exposure to sunlight will maintain the green color (Mohr & Wehrle 1942). However, sunlight will most probably start deterioration processes, which will become obvious many years later. A medium composed of camphor, isobutyl alcohol, phenyl salicylate, and gum sandarac as well as the recipe by Mohr & Wehrle (1942) agreed widely with the recipe for Euparal (Tab. 5; Spence 1940a).

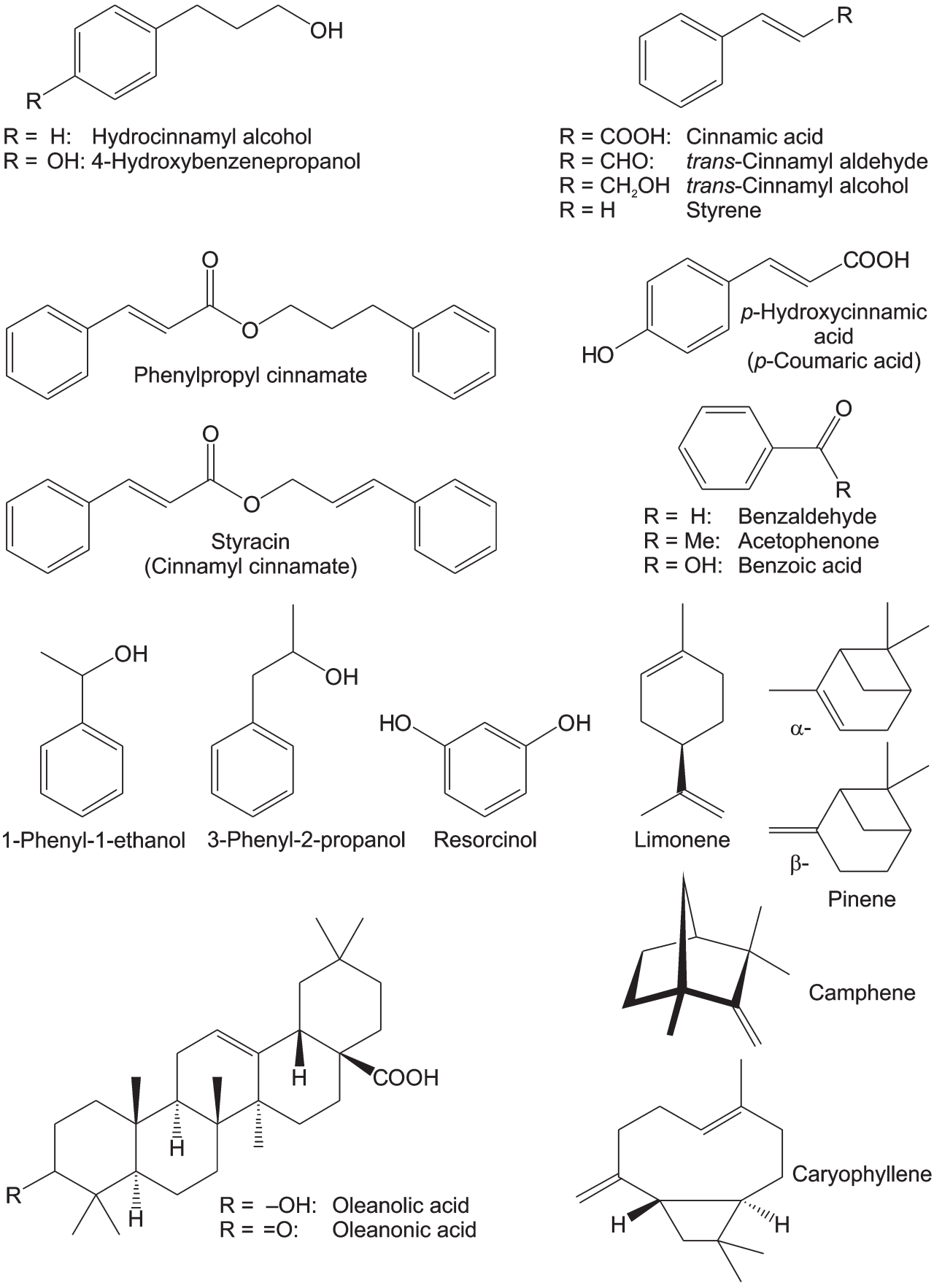


FIGURE 9. Chemical structure of the potential ingredients of styrax or storax resin from *Liquidambar orientalis*. Me, methyl.

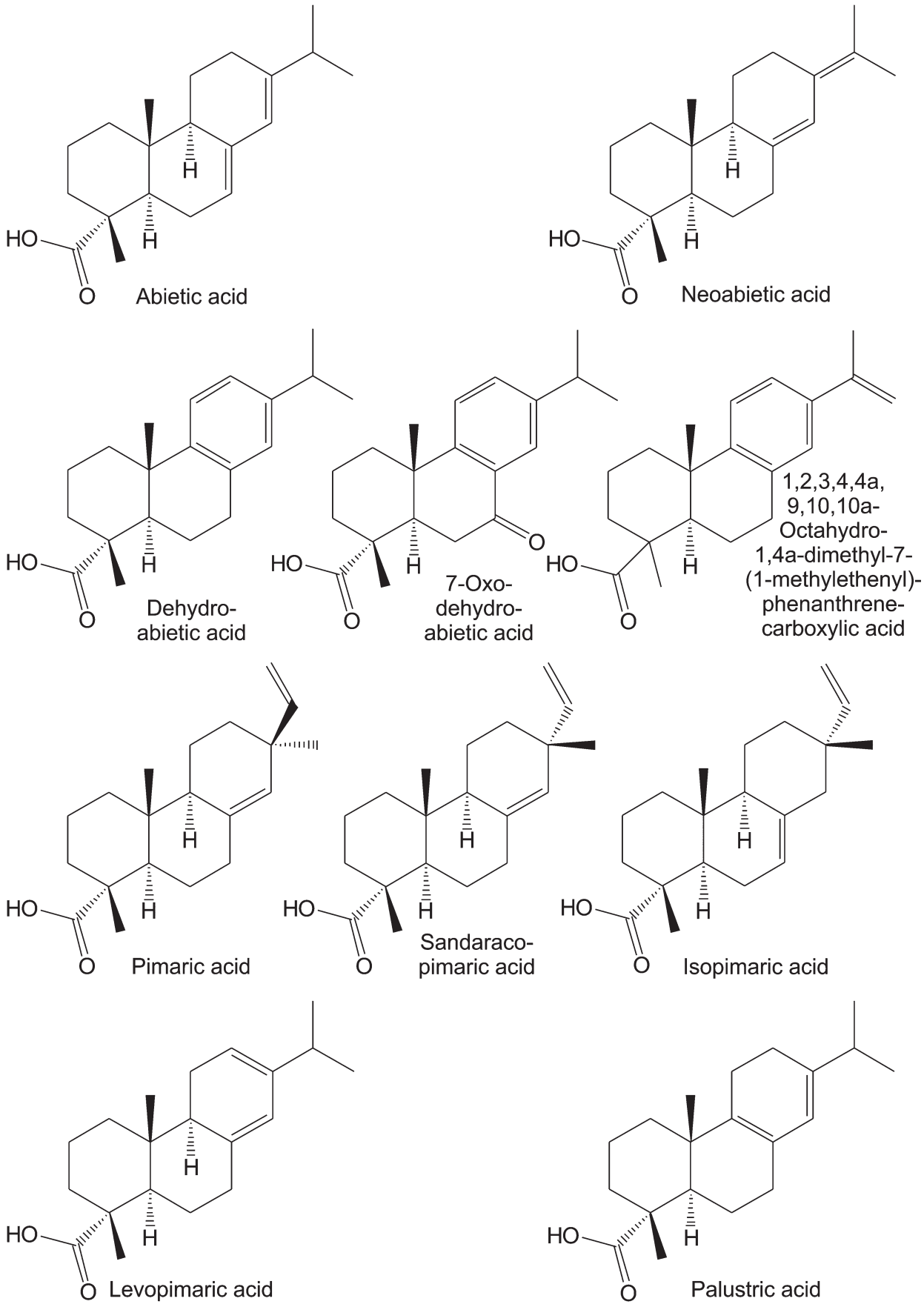


FIGURE 10. Chemical structure of the resin acids of Venetian turpentine from *Larix decidua*.

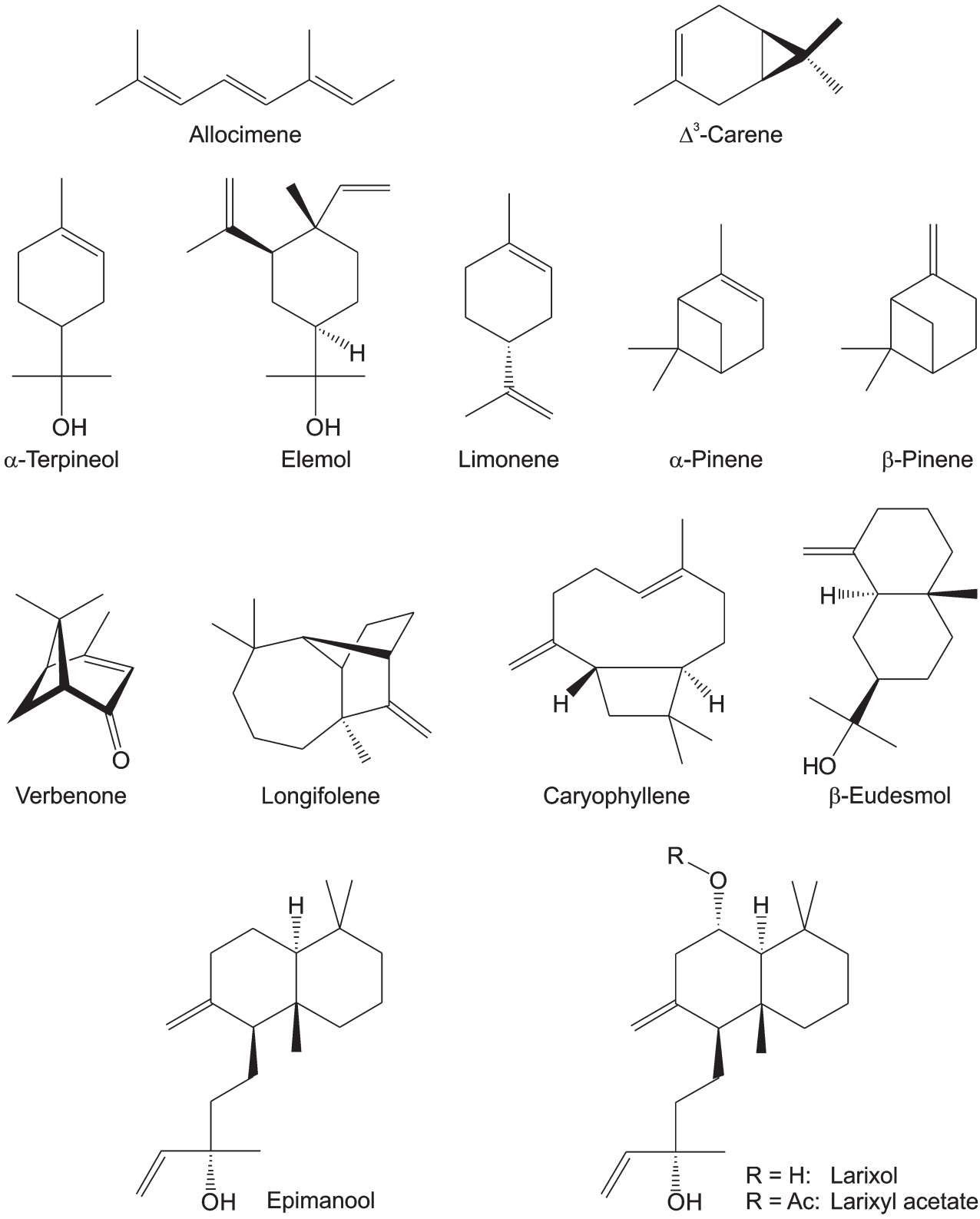


FIGURE 11. Chemical structure of the more volatile ingredients of Venetian turpentine from *Larix decidua*. Ac, acetyl.

3.7.13 Glycerol-gelatin

Glycerol-gelatin (= glycerol-jelly) was first used in 1852 by Deane (Tab. 1; Bracegirdle 1978, p. 93). It may serve as mounting medium and consists of gelatin, glycerol, water, and in the cases of Kaiser's glycerol-gelatin and

Niglytin also phenol (Tab. 5; Amann 1896; Beck 1963; Jentzen 1986; Maybury *et al.* 1991). Niglytin contains the stain nigrosin, which creates a dark background and, therefore, allows visualization of structures with a poor contrast including the mucous of algae (Jentzen 1986). A stain included in the mounting medium will migrate to the specimen, which will be surrounded by a clear "halo" that is medium with depleted stain (Denham 1923, p. 192). A medium similar to Kaiser's glycerol-gelatin contains gelatin, glycerol, lactic acid, phenol, and water (Amann 1896). Zander (1997) used only a low amount of phenol or thymol in order to avoid growth of fungi and bacteria. It can be expected that phenol will evaporate soon from any un-ringed mount because of its low vapor pressure (Tab. 10). Zirkle (1937) suggested a one-step fixing, staining (carmine), and mounting medium with a more complex composition (Tab. 5). After boiling, the medium became very firm and could not be re-liquefied again by heating to 80°C. Subsequently, it turned out that the medium suffered from various problems (Tab. 6), and Zirkle (1940) replaced it by a mixture of glacial acetic acid, carmine, ferric nitrate ($Fe(NO_3)_3$), gelatin, sorbitol, and water. Amann (1896) suggested replacement of lactophenol by copper chloride and copper acetate for plant material.

Specimens are mounted directly from water on a glass slide warmed on a hotplate, which allows control of the temperature more accurately than a Bunsen burner; to this slide, a piece of the solid medium is added and covered by a warmed coverslip (Romeis 1948, p. 192; Evens 1961; Beck 1963; Adam & Czihak 1964; Steedman 1976c, pp. 190–191; Jentzen 1984; Hevers 1985; Maybury *et al.* 1991). Sanderson (1994, pp. 154–155) recommended mounting a specimen from glycerol instead from water in order to prevent cracking of the medium. Amazingly, the temperature suggested for heating varies considerably from 40°C (Romeis 1948, p. 192; Steedman 1976c, p. 190), 50°C (Maybury *et al.* 1991), 80°C (Beck 1963), to even 100°C (Wilson 1971). The coverslip must be sealed (Evens 1961; Beck 1963; Jentzen 1984; Hevers 1985; Maybury *et al.* 1991). Peripheral glycerol-gelatin may be hardened with about 4% formaldehyde before ringing (Beck 1963). Some authors report that glycerol may separate from the gelatin and the coverslip may detach (Evens 1961; Steedman 1976c, pp. 190–191; Maybury *et al.* 1991). This is supposed to be avoided if 5% formaldehyde or 1% chrome alum are added immediately before mounting. In this way, the gelatin is fixed, and the mount cannot be melted again (Evens 1961; Loveland & Centifanto 1986, p. 211; Zander 1997). Phenol may fade stains, so thymol is suggested as a preservative (Sanderson 1994, p. 192). Fungi are assumed to invade glycerol-gelatin mounts if not sealed with Caedax, but evidence is not provided (Frahm 1990).

Gatenby & Beams (1950, p. 209) and Mitchell & Cook (1952) claimed that mounts might last at least 50 years, but Woessner (2005) suggested a life time not beyond 10 years, before the medium cracked. Re-mounting of the preparation is done by heating the slide again. However, this procedure should not be repeated too often, because the medium will not become liquid; if the temperature rises too high or if the medium is kept liquid for more than one week, the medium will not harden again (Morrison 1942; Beck 1963; Hevers 1985; Loveland & Centifanto 1986, p. 211; Sanderson 1994, p. 192; Zander 2014). If glycerol-gelatin has solidified around a specimens or remains on the slide, soaking in a 10% trypsin solution at 20°C for 24 hours may assist in freeing a paleontological specimen (Green 1995, p. 162). However, the trypsin may digest a recent biological specimen. Glycerol-gelatin may also form cavities, and the coverslip may crack above the specimen (Fig. 18A).

3.7.14 Gum-chloral media

Gum-chloral media are named after the author, who supposedly first introduced the respective recipe, e.g., Berlese, Davidson, Doetschman, Ewing, Faure, Hoyer, Imms, and Stroyan, but these assignments seem to be erroneous and misleading in many cases (Upton 1993, pp. 267–272, tab. 1). For example, the earliest record by Hoyer (1882, pp. 23–24; Tab. 1) was quite vague about an exact formula, thus Faure (1910) presented the first precise recipe of a gum-chloral medium. One of the more famous known media, Berlese's medium lacking glycerol, seems to have never been used by Antonio Berlese; instead he mounted his slides in Hoyer's mounting medium containing glycerol (Upton 1993, p. 269). Gum-chloral media are still not commercially available but mixed in the scientist's laboratory, possibly also, because chloral hydrate is now a controlled substance in several countries.

Gum-chloral media comprise water-soluble media with or without glycerol ("Berlese"-type), with a low or a high chloral hydrate content, with or without glucose syrup or sorbitol, and with or without acetic acid (Upton 1993, tab. 1). The media contain gum arabic, which consists *inter alia* of glucuronic acid forming a salt with calcium, magnesium, and potassium (Dahl 1951; Mills & White 1999, p. 77; Dror *et al.* 2006). Such media also

contain glycoproteins and polysaccharides from the gum arabic and in some recipes added sugars (Tab. 5; Upton 1993, tab. 1; Brown 1997, pp. 1416; Mills & White 1999, p. 77; Dror *et al.* 2006). Chloral hydrate (see chapter 3.1.2 Chemical maceration) represents a major constituent of any gum-chloral medium. Although solid, chloral hydrate has a high vapor pressure (Tab. 10) and may evaporate from an exposed medium in the periphery of the mount over a period of several years (Schmid *et al.* 2016) and from stock solution (Stock & Vaupel Klein 1996). Glycerol is supposed to function as plasticizer (Lillie *et al.* 1953, p. 73). Iodine and potassium iodide in gum-chloral media result in enhanced contrast of cuticular structures (Higgins 1971, 1986; Westheide & Purschke 1988; Amrine & Manson 1996; Faraji & Bakker 2008). Thiomersal (= thimerosal, trade name: Merthiolate; contains mercury), phenol, thymol, or white arsenic (As_2O_3) are occasionally added as protection against biological growth (Lillie *et al.* 1953, p. 75).

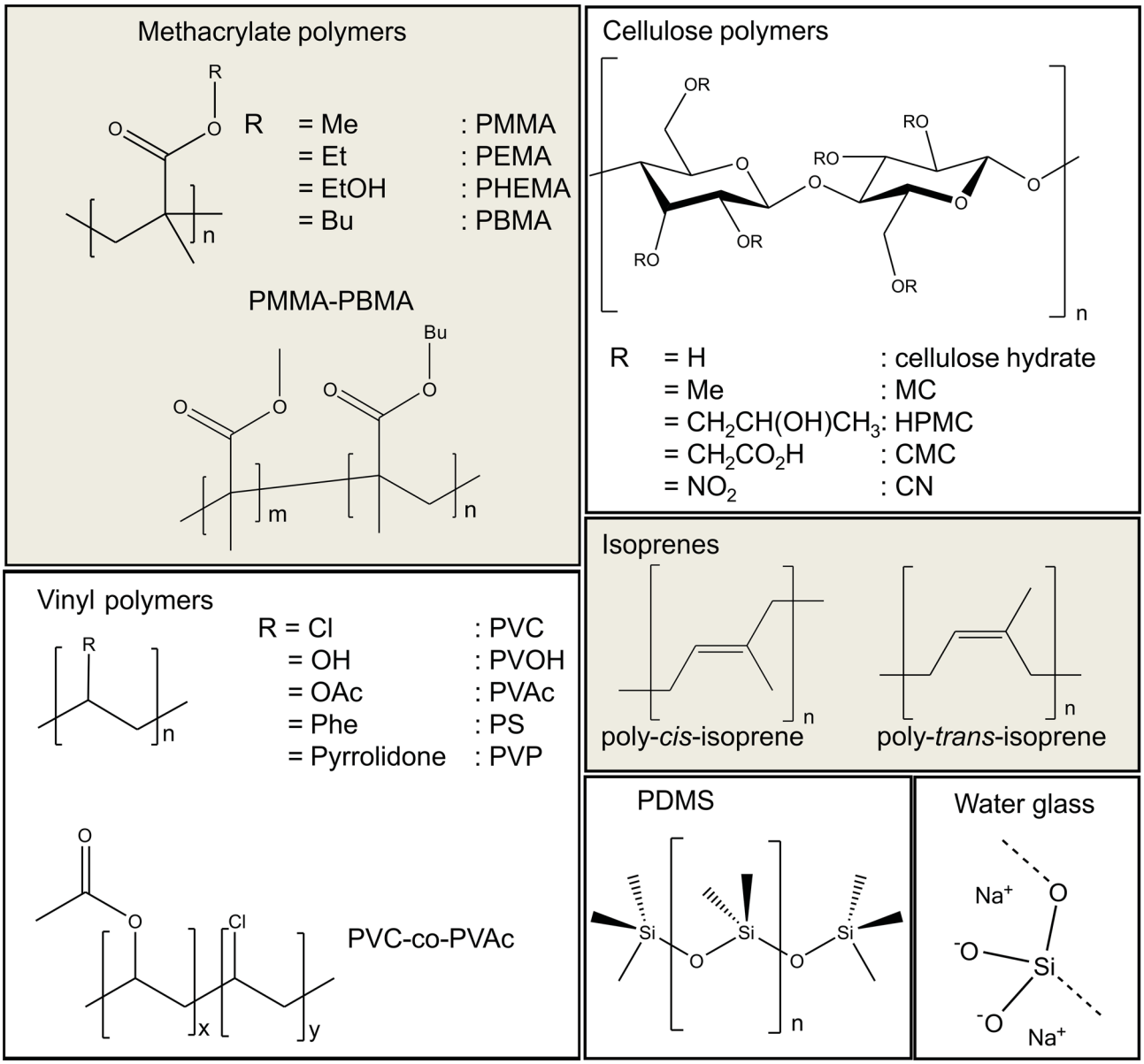


FIGURE 12. Chemical structure of various synthetic polymers. Bu, butyl; Cl, chlorine; CMC, carboxy methyl cellulose; CN, cellulose nitrate; Et, ethyl; EtOH, hydroxyethyl; HPMC, hydroxypropyl cellulose; MC, methyl cellulose; Me, methyl; OAc, hydroxyacetyl; PBMA, poly(butyl methacrylate); PDMS, poly(dimethyl siloxane); PEMA, poly(ethyl methacrylate); Phe, phenyl; PHEMA, poly(2-hydroxyethyl methacrylate); PMMA, poly(methyl methacrylate); PMMA-PBMA, copolymer of PMMA and PBMA; PS, polystyrene; PVAc, poly(vinyl acetate); PVC, poly(vinyl chloride); PVC-co-PVOAc, copolymer of PVC and PVAc; PVOH, poly(vinyl alcohol); PVP, poly(vinyl pyrrolidone).

Gum-chloral media deteriorate over time and reveal precipitations, segregation of their components, cavities, crystallization, blackening, opaqueness, and excessive maceration to a point, where a specimen is not visible anymore (Tabs 6, 7; see also chapter 3.7.25 Discoloration and chapter 3.10 Restoration procedures; Swan 1936; Kabata 1986; Upton 1993; Amrine & Manson 1996; Brown 1997, pp. 9–10; Lillo *et al.* 2010; Neuhaus & Kegel 2015; Walter & Krantz 2009; Neuhaus pers. obs.). Parasitic Copepoda, stored in a preservative fluid for more than 120 years, have been mounted in Berlese's mounting medium just for re-examination; however, specimens were macerated by the medium within days and could not be traced again (Kabata 1986). This author attributed the disintegration of the specimens to the age of the specimen and to the unknown pre-treatment. Too intense maceration of a specimen originates from the chloral hydrate, which exists in any gum-chloral medium to a variable amount (Upton 1993; Brown 1997). Maceration of a specimen is stated to be slowed down by integrating formaldehyde, which hardens the tissue in the formula of the mounting medium (Jeppson *et al.* 1975, p. 389). The same effect can be observed in specimens stained with Rose Bengal to facilitate easier sorting, which still reveal most of the internal organs after decades (Neuhaus pers. obs.). Several authors recommended reducing the amount of chloral hydrate (Swan 1936; Higgins 1977, 1982, 1983, 1988; Frahm 1990).

Fine background granulation and segregation of its chemical components occur at least in Hoyer's mounting medium (Neuhaus 2013, fig. 5.5.2C; Neuhaus pers. obs.). Possibly, the crystals observed in all gum-chloral media form on drying of the mounting media either from chloral hydrate, from evaporation of acetic acid, from the calcium and magnesium salts of the glucuronic acid of the gum arabic, from the polysaccharides of the gum arabic, or from a combination of several components (comp. also Tab. 7). Consequently, Dahl (1951, p. 99) suggested precipitating the calcium and magnesium ions with sodium carbonate before using the medium. Swan (1936) and Higgins (1983) reduced the amount of chloral hydrate in the medium. Swan (1936) recommended re-dissolving the crystals by adding some glacial acetic acid to the periphery of the mount and by subsequent heating the slide until boiling. Lillie *et al.* (1953, p. 73) concluded from experimental slides with very high concentrations of glucose, invert sugar, maltose, sucrose, and white Karo crystallizing within a month that sugars were responsible for crystallization of gum-chloral media. These sugars were added to some types of gum-chloral media but not to all in order to raise the refractive index of the media (Lillie *et al.* 1953, p. 73). Crystallization of media was also attributed to variable quality of gum arabic possibly sampled from different *Acacia* trees (Tab. 7; Freeman 1987).

Quite conflicting opinions exist as to whether microscope slides with gum-chloral media should be ringed or not and whether or not this would inhibit crystallization of the mounting medium (Tab. 7; summarized by Upton 1993, pp. 267–272). Crystallization has been experienced early until recently (Tab. 7) and different hypotheses have been proposed about the origin of these crystals (Tab. 7). The controversial opinions about the advantages and disadvantages of gum-chloral media are summarized by date in Table 7 in order to allow the reader to make up his/her mind about the rationale behind the arguments given in the literature despite the ever growing evidence for the disastrous properties of gum-chloral media. Upton (1993, p. 273) summarized "The reasons for the many cases of deterioration being encountered appear to be numerous and varied. Incursions of air, too much chloral hydrate, too little chloral hydrate, crystallization of chloral hydrate, poor quality gum arabic, presence of phenols, absorption of atmospheric moisture, too much glucose, contamination of medium by ringing compound, incorrect heat treatment of slide after preparation, etc., have all been cited as causes. To complicate matters still further the deterioration often occurs randomly within a single batch of slides with some breaking down a year or more ahead of others. Eventual breakdown would now, however, appear inevitable." He also strongly suggested not to mount any specimen of taxonomic importance in a gum-chloral medium and to use Euparal instead, because its refractive index was close to that of the gum-chloral media and its longevity was well known. Probably, the regulations about chloral hydrate in some countries may solve the dispute about using gum-chloral media in the long run, but the problem with existing deteriorating collections will continue for quite some time.

Keifer (1979, p. 23) and Amrine & Manson (1996, p. 386, tab. 1.6.3.1) suggested replacing gum arabic of gum-chloral media by benzophenone- 3,3',4,4'-tetracarboxylic dianhydride (= BTDA; Fig. 14; Tab. 5) for mounting mites. No written information was found about how long slide mounts may last in this medium. However, Amrine (pers. com.) mentioned problems with too intense maceration of specimens and formation of crystals in the medium but not with granulation of the medium, which is common in gum-chloral media (Tab. 6). Amann (1896) used gum arabic, glucose, and lactophenol.

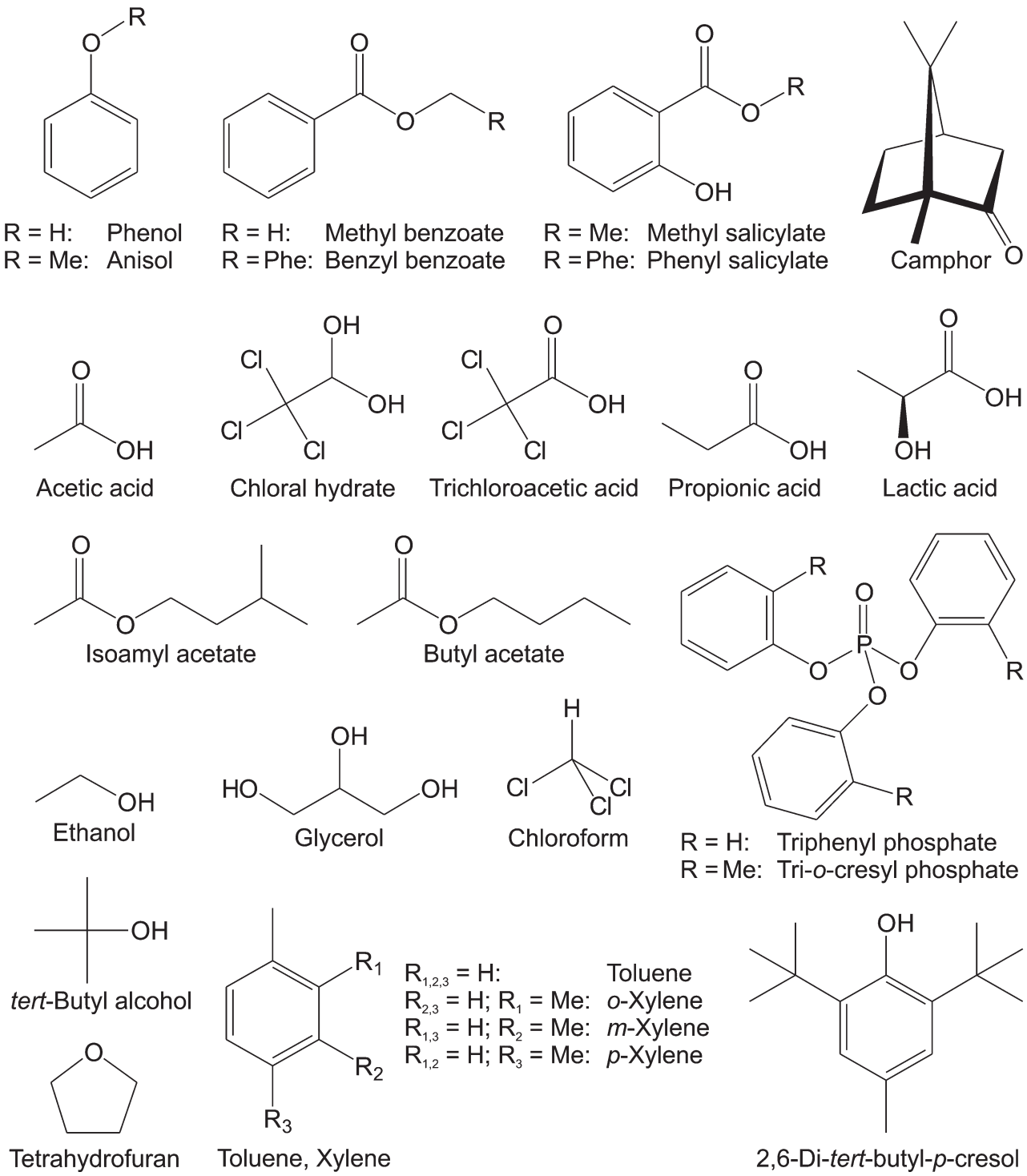


FIGURE 13. Chemical structure of various chemicals such as clearing agents, macerating agents, plasticizer, and organic solvents used for microscope slide preparation, part 1. Me, methyl; Phe, phenyl.

3.7.15 Permount™

Permount™ seems to have been produced in at least two formulae, and there is some confusion in the literature about its composition. The original formula is supposed to represent a naphthalene polymer and to have become unavailable before 1953 (Lillie *et al.* 1953, p. 66). Wicks *et al.* (1946, p. 122) claimed that this Permount™ (old) was identical with Clarite X (= Nevillite I) of Groat (1939), which became unavailable before 1950 (Lillie *et al.* 1950, p. 2). However, Lillie *et al.* (1950, p. 4, 1953, tab. 1B) stated that Clarite and Clarite X were cycloparaffin

polymers. Roe *et al.* (1991, p. 62) mentioned that "Permount has been reformulated in recent years", which may indicate a third formula, because these authors probably did not refer to the change before 1953.

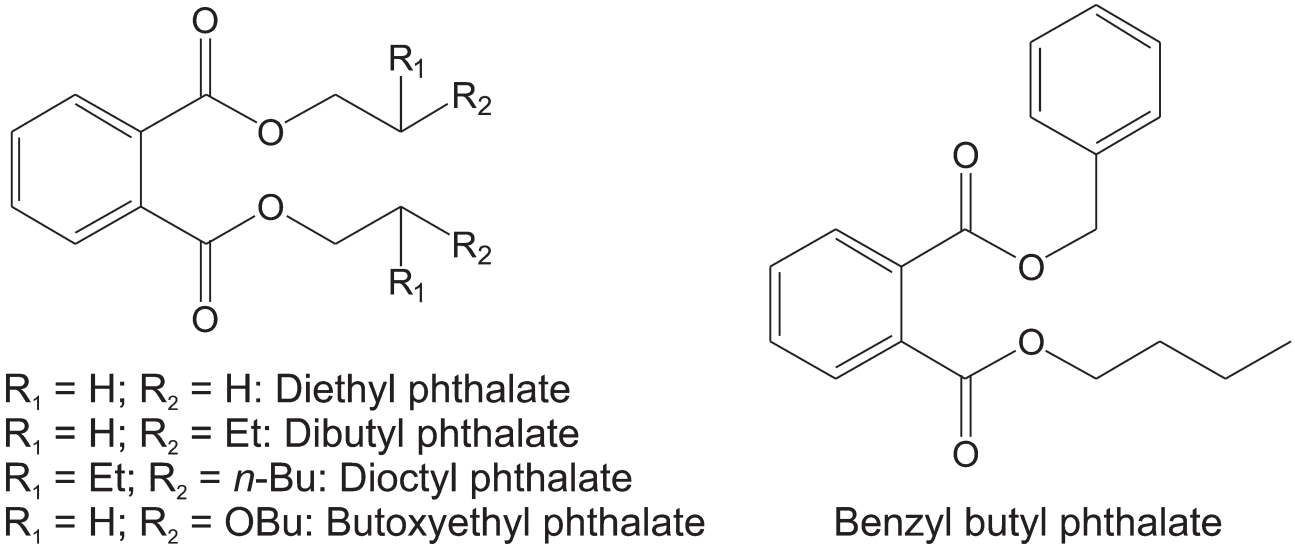


FIGURE 14. Chemical structure of various chemicals such as clearing agents, macerating agents, plasticizer, and organic solvents used for microscope slide preparation, part 2. Et, ethyl; *n*-Bu, *n*-butyl; OBu, butoxy.

Permount™, probably the new formula mentioned by Lillie *et al.* (1953), is reported to develop annular bands of cracks and to exhibit fading stains; both processes are assigned to oxidation starting from the margin of the coverslip (Figs 16D–J, 17A–F; Hollander & Frost 1970, 1971; Loveland & Centifanto 1986, p. 189; Halliday 1994). An anti-oxidant like 2,6-di-*tert*-butyl-*p*-cresol has been found to inhibit both cracking and fading for at least eight years (Hollander & Frost 1971). One reviewer of this manuscript mentioned that N. W. Riser added terpineol at 5% to Permount™ therefore increasing the longevity of the mountant to decades. This concentration is also found in Bullock (1980). An unknown anti-oxidant is used in today's formulae at least by Bioworld and EMS (see references in Tab. 5). Crystals form within 5–20 years on microscope slides with Lepidoptera at the Natural History Museum London, but it seems the problem can be overcome in this case by soaking the slide in xylene (Halliday 1994; Brown 1997, p. 9).

However, this procedure does not work that successfully with mounts of the kinorhynch *Cateria gerlachi* Higgins, 1968. The mounting medium of almost all slides is impacted by deterioration, which at an early stage forms irregularly distributed narrow cracks in the otherwise transparent medium (Fig. 16G–I) and, at later stages of deterioration, develops more and wider cracks and turns whitish to yellowish macroscopically (Fig. 16D–F, J, 17A–F; Neuhaus & Kegel 2015, fig. 18A–C). Specimens cannot be studied with light microscopy anymore once the cracking has begun. Cracking starts initially in the entire medium (Fig. 16G–I), and may form wider cracks more or less from the periphery of the coverslip towards the center (Fig. 16J), but may leave some peripheral areas

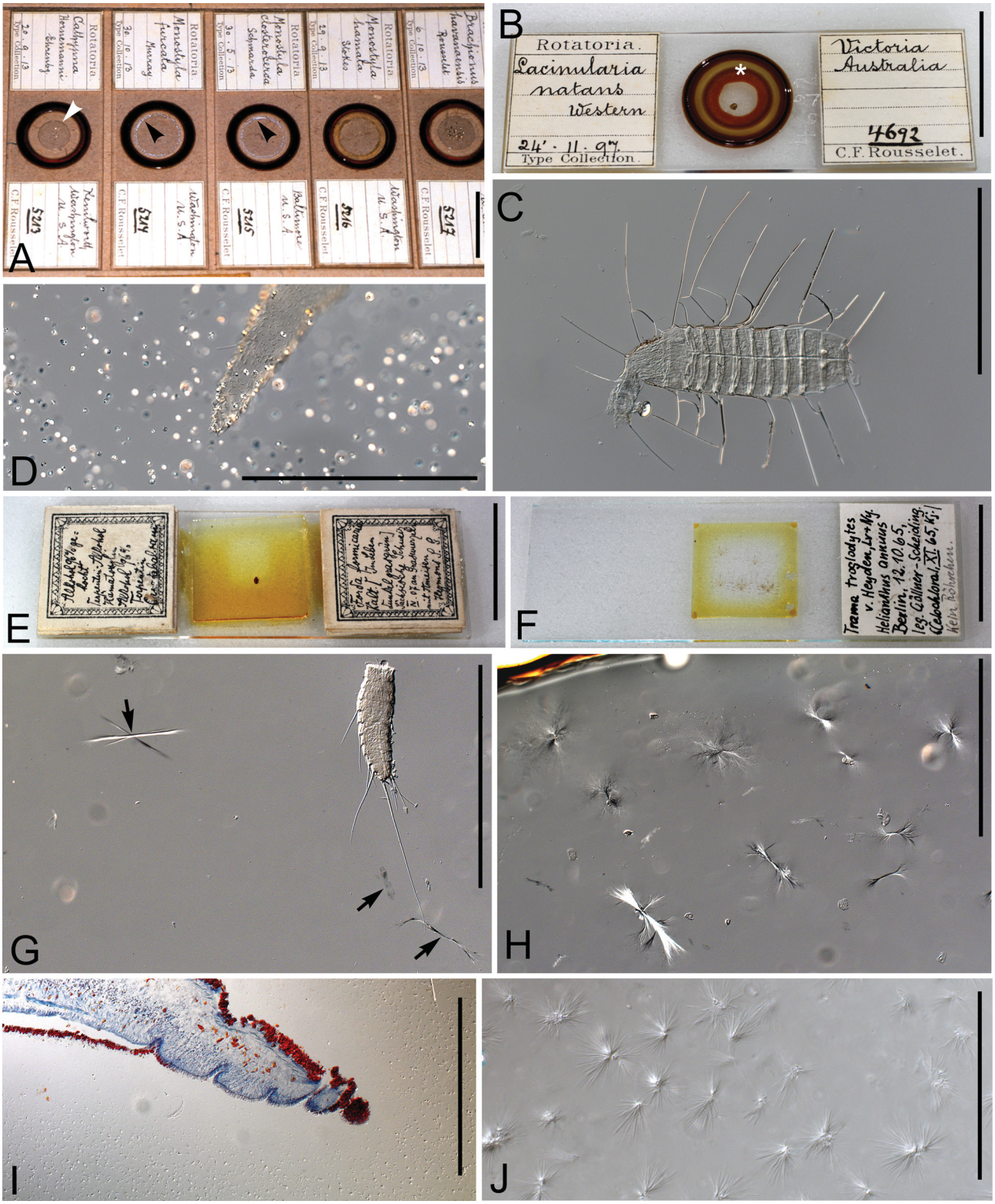


FIGURE 15. A, B. Liquid mounts, mostly dry except for 1st slide in **A** with central gas bubble (white arrowhead) and **B**; mounted probably in formaldehyde about 1913 (**A**) and about 1897 by Rousselet (**B**). Notice precipitations in **A** (black arrowheads) and inner coverslip seal of foamy brownish structure (**B**, asterisk). **C.** Kinorhynch mounted in Aquatex® between 1985 and 1990 by Neuhaus showing cracks in the medium. **D.** Part of Aphidina mounted in Caedax with numerous small bright crystals. **E, F.** Aphidina mounted in Canada balsam 1907 by Heymont (**E**) and in Celochloral in 1965 by Göllner-Scheiding (**F**). Notice yellowed mounting medium in periphery of coverslips. **G, H.** Kinorhynch mounted in CMCP-10 in 1992 by Neuhaus. Notice crystals near specimen (arrows in **G**) and in periphery of coverslip (**H**). **I, J.** Histological sections mounted in DPX in the 1980s in the lab of Sluys. Notice crystals at a distance from section (**I**), enlarged in **J**. **A**, **B**, **E**, **F**: macro lens; **C**, **D**, **G-J**: DIC. Scalebars: **A**, **B**, **E**, **F** 2 cm; **C, G-I**, 500 µm; **D**, 300 µm; **J**, 50 µm.

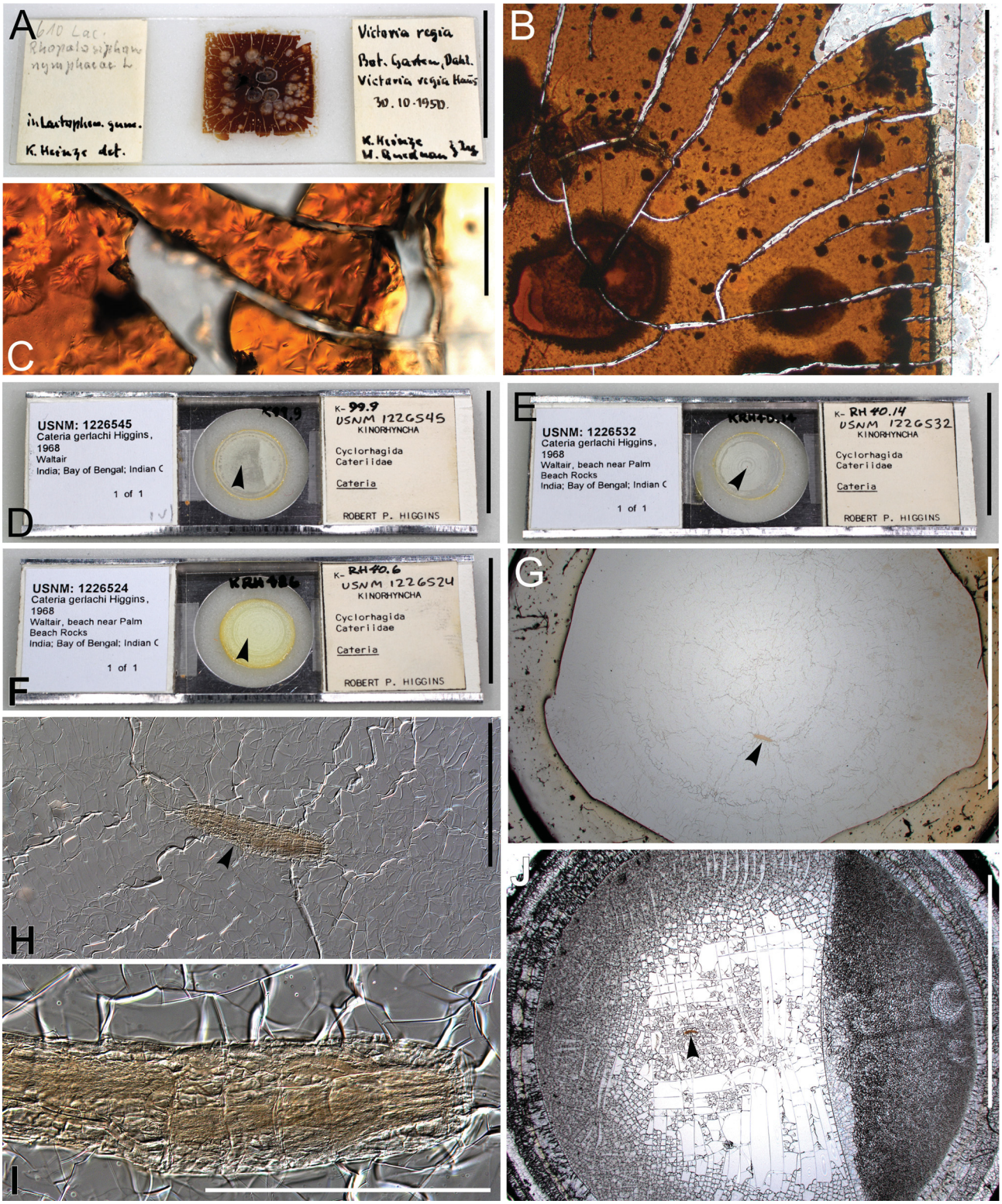


FIGURE 16. A-C. Aphidina mounted in lactophenol gum about 1950 by Heinze, overview (**A**) and enlargements with cracked and darkened medium (**B**, **C**) with crystals (**C**). **D-J.** Kinorhyncha mounted in Permount™ between 1964 and 1968 by Higgins; early stage of deterioration with initial cracks (**G-I**), intermediate stage with incomplete crack coverage (**D**, **J**: see also same slide in Fig. 16A), and final stages with entirely cracked and whitish (**E**: see also same slide in Fig. 16B, C) or yellowish (**F**: see also Fig. 16D-F; same slide) mounting medium. Arrowheads mark specimens. **A**, **D-F**: macro lens; **B**, **G**, **J**: bright field illumination; **C**, **H**, **I**: DIC. Scalebars: **A**, **D-F**, 2 cm; **B**, **G**, **J**, 5 mm; **C**, **H**, 300 µm; **I**, 200 µm.

with narrower cracks (Fig. 17B). In two cases with no or only peripheral cracks, the upper coverslip detached during examination with an oil immersion lens. Restoration efforts with soaking in water and thymol crystals added to prevent bacterial and fungal growth for more than 10 weeks on a hotplate at 45° C were totally in vain as were soaking tests with solvents like ethanol and acetone. Eventually, xylene proved to dissolve the medium within less than a minute resulting in specimens broken into several pieces or at least without many of the spines or with broken spines (Neuhaus & Kegel 2015). Probably either the cracks in the mounting medium have destroyed the specimen during their formation, or the solvent xylene causes a rapid swelling of the polymer leading to the breakdown of the specimen's integrity. A small part of the old mounting medium seems to remain partly with the specimen (Neuhaus & Kegel 2015). The mounting medium of *C. gerlachi* certainly did not represent Hoyer's medium as stated in the original publication (Higgins 1968), also because the large cracks did not resemble the crystals that the senior author has seen in other specimens mounted in Hoyer's. Raman spectroscopy revealed that Permount™ was used (Schmid *et al.* 2016), a medium that Higgins mentioned in an earlier publication for a different species (Higgins 1961). From this discovery it can be concluded that Higgins applied Permount™ for mounting kinorhynchs on microscope slides until at least 1967. Our results on *Cateria gerlachi* mounted in Permount™ agree entirely with the findings of Hollander & Frost (1970, fig. 1A–D) on cytopathological slides mounted with Permount™ and Histoclad®. These authors suggested seasonal fluctuations in temperature, humidity, etc. as cause of the regularly arranged peripheral cracking.

3.7.16 Polyacrylates and polymethacrylates

Polyacrylates. A water-soluble acrylic varnish for household purposes, "Tiger neu Email wasserlöslicher Acryllack" (Available from: http://www.tiger.at", accessed 17 December 2015), was suggested for parts of insects as a possible temporary mounting medium, because a specimen could be mounted directly from water in the varnish, which saved quite some time; the mounts lasted at least two years (Lödl 1999). The varnish is milky on application, but dries to a transparent medium if the specimen is not too thick. Unfortunately, it turned out that the medium shrank to an extent that extensive cavities developed in the medium (Lödl pers. com.). The acrylate varnish is derived from an acrylic acid (Horie 2011, p. 153).

Polymethacrylates. Polymethacrylates (Fig. 12; Tab. 5) are components of mounting media like Entellan® new [PnBMA = poly(*n*-butyl methacrylate)], Eukitt™ [PBMA-PMMA = poly(butyl methacrylate-co-methyl methacrylate)], Elvacite® 2044 [previously Lucite® 44; PnBMA = poly(*n*-butyl methacrylate)], Elvacite® 2046 [previously Lucite® 46; PnBMA-PiBMA = poly(*n*-butyl methacrylate-co-*iso*-butyl methacrylate)], Loctite® 363™ Impruv® Potting Compound Light Cure (methacrylate, polyurethane methacrylate resin, hydroxyalkyl methacrylate, g-glycidoxypropyl trimethoxysilane), Lucite™ [PMMA = poly(methyl methacrylate)], Technovit® 7100 [PHEMA = poly(2-hydroxyethyl methacrylate)], and for light and electron microscopy such as HPMA (= poly(2-hydroxypropyl methacrylate) (Richards & Smith 1938; Frison 1955; Leduc & Holt 1965; Feller 1971, p. 122; Crumpton 1987; Horie 2011). Loctite® 363™ Impruv® Potting Compound Light Cure is cured by UV light (Silverman 1986). A comparison of four MSDS of this medium from 2004, 2007, 2010, and 2014 reveals changes in the ingredients by the manufacturer (Tab. 5). Pontalite, introduced as mounting medium by Skiles & Georgi (1937), is supposed to be either a polystyrene (Gray & Wess 1951) or identical with the poly(methyl methacrylate) Lucite™ (Lillie *et al.* 1950). Technovit® 7100 and HPMA require a hardener and can be stored mixed with the hardener in a freezer for at least one year; polymerization may be at 60°C or by UV light (Tab. 5; Leduc & Holt 1965; Crumpton 1987; Sänger pers. com.).

Both poly(methyl methacrylate) and poly(*iso*-butyl methacrylate) do not qualify as a permanent mounting medium if used alone, because they do not adhere well to glass surfaces and some stains fade within less than five months (Richards & Smith 1938; Groat 1940; Hamilton 1940; Loveland & Centifanto 1986, p. 192). At least poly(methyl methacrylate) sets on drying and cavities develop in the periphery of the coverslip (Richards & Smith 1938), but its UV-durability seems to be satisfying if stabilizers are incorporated into the polymer (Wypych 2013, p. 358). Diffusion of water causes stress cracking of the polymer over time (Wypych 2013, p. 429). Therefore, Groat (1950) advocated a copolymer of *iso*-butyl methacrylate and styrene, whereas Loveland & Centifanto (1986, p. 192, tab. III) suggested several different plasticizers for various methacrylates (Tab. 14), e.g., diethyl phthalate for poly(methyl methacrylate), dibutyl phthalate for poly(ethyl methacrylate), butoxy ethyl phthalate for poly(*n*-butyl methacrylate), and castor oil for poly(*iso*-butyl methacrylate). These authors also stated that certain stains

faded in poly(methyl methacrylate-co-butyl methacrylate), except if the amount of the peroxide catalyst was reduced to far less than 1%. Commercial products are supposed to contain the catalyst well in excess in order to compensate for an inhibitor in the raw mixture without actually having to remove the inhibitor (Loveland & Centifanto 1986, pp. 192–193). As picture varnishes, both poly(*n*-butyl methacrylate) and poly(*iso*-butyl methacrylate) "develop 50–80% insoluble matter after exposure" to "daylight under an average illumination level of 50 footcandles" for 50 years (Lomax & Fisher 1990). These methacrylates can be removed from pictures even after about 30 years with acetone, toluene, or mixtures thereof. In histology, methacrylates may be dissolved in acetone or in sodium ethoxide made from sodium hydroxide in anhydrous ethanol (Sanderson 1994, p. 72). In another recipe, sections of poly(methyl methacrylate-co-butyl methacrylate) are soaked in 2-methoxy ethyl acetate and subsequently in acetone (Erben 1997).

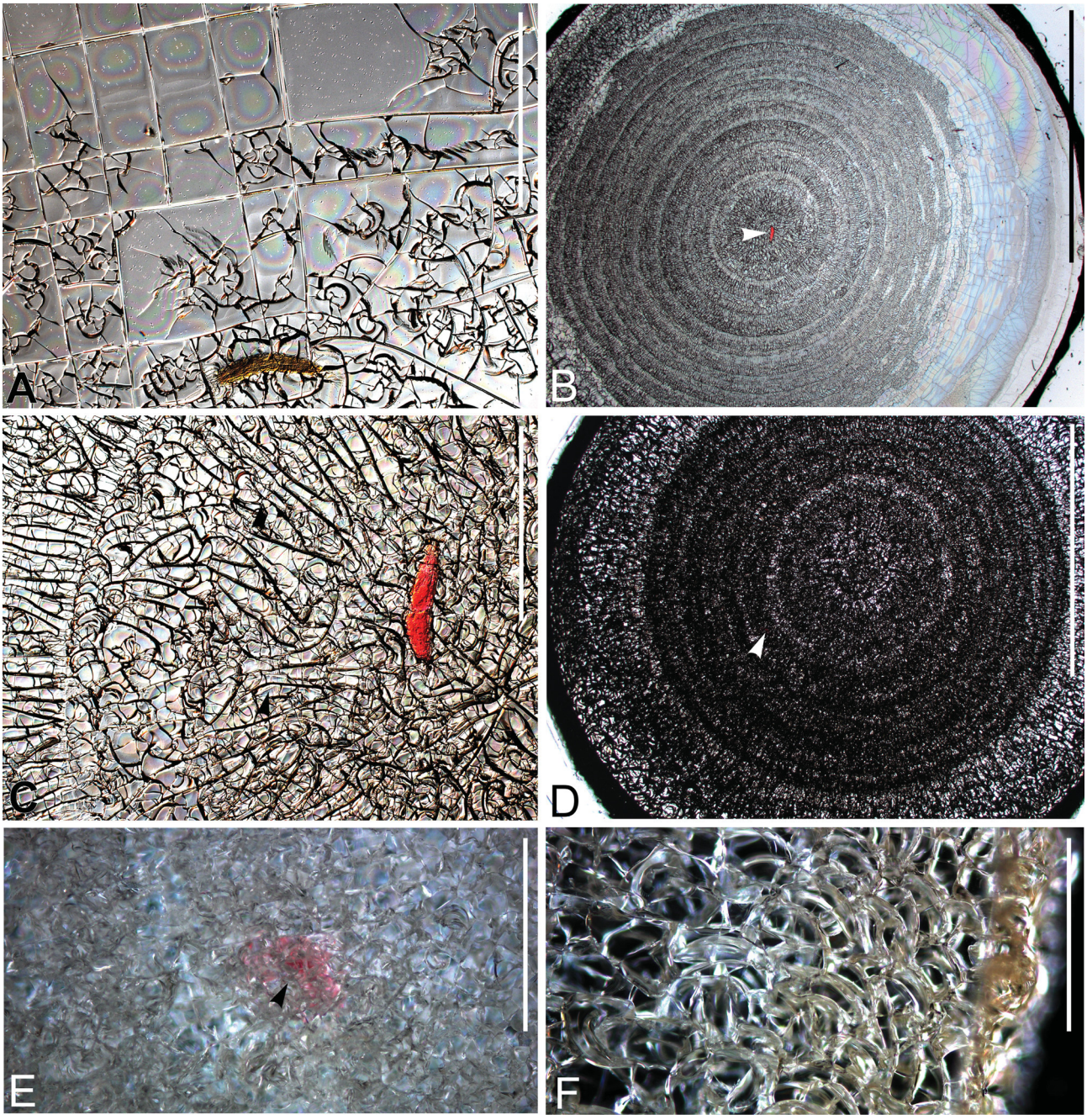


FIGURE 17. A-F. Kinorhyncha mounted in Permount™ between 1964 and 1968 by Higgins; intermediate stage of deterioration with incomplete crack coverage (**A**: see also same slide in Fig. 15J), and final stages with entirely cracked and whitish (**B**, **C**: see also same slide in Fig. 15E) or yellowish (**D-F**: see also same slide in Fig. 15F) mounting medium. Cracks at specimen (**E**) and at margin of coverslip (**F**). Arrowheads mark specimens. **A**, **C**: DIC; **B**, **D**: bright field illumination; **E**, **F**: dark field illumination. Scalebars: **A**, **C**, **E**, **F**, 500 µm; **B**, **D**, 5 mm.

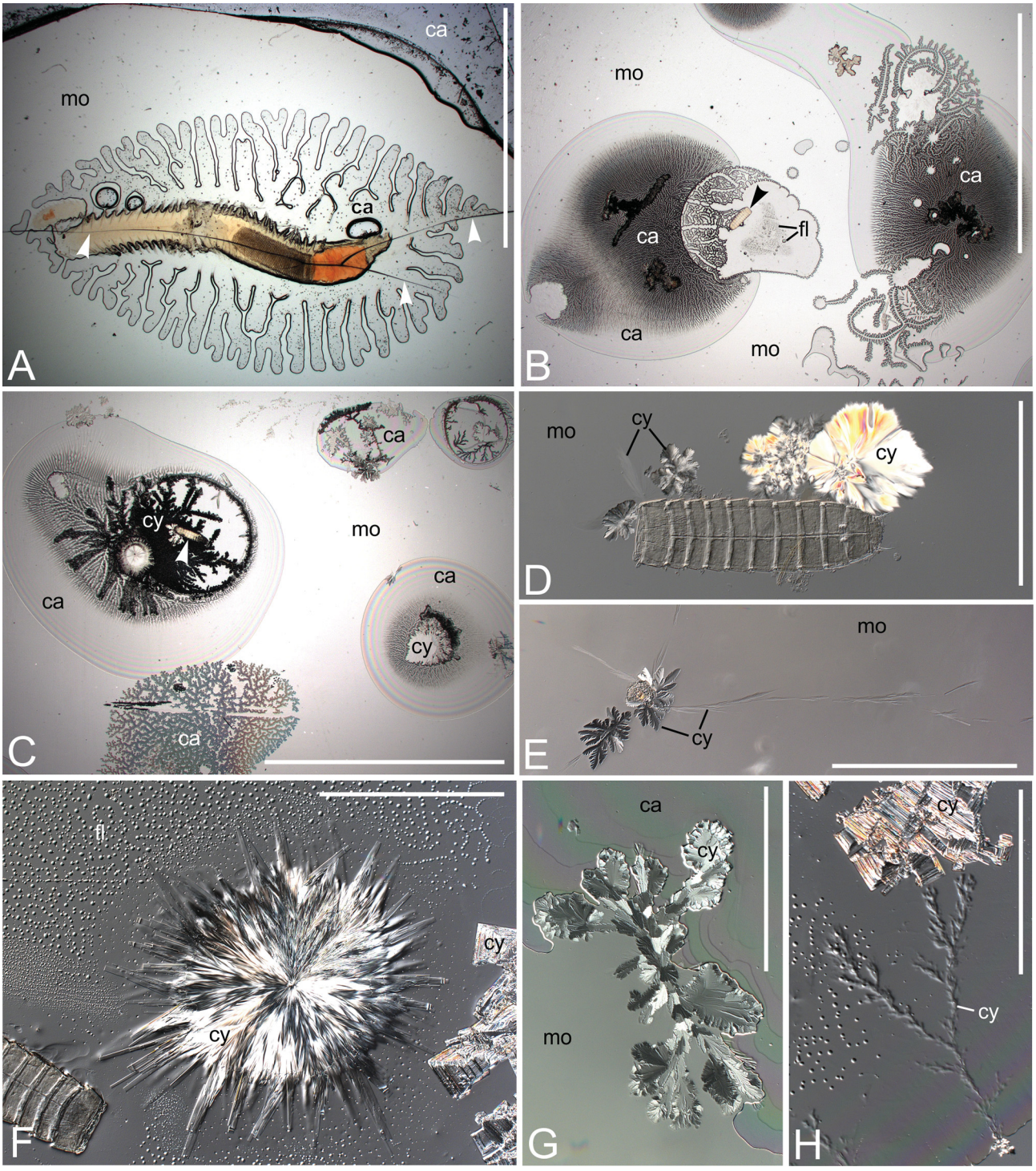


FIGURE 18. A. Polychaete in glycerol-gelatin with unharmed medium (mo), formation of cavities (ca), and coverslip cracked above specimen (arrowheads). **B-H.** Kinorhyncha mounted in polyvinyl lactophenol between 1985 and 1990 by Neuhaus, unringed; currently not affected mounting medium (mo) and areas at different stages of deterioration with extensive cavities (ca), drops of fluid (fl; **B**, **F**), and formation of different kinds of crystals (cy). **B, C.** Overview of damage on two slides. Arrowheads mark specimens. **D-F.** Details of radially growing (**D**, **F**), rectangularly growing (**F**, **H**), leaf-like (**D**, **E**, **G**), and tree-like (**H**) crystals. **A-C**: bright field illumination; **D-H**: DIC. Scalebars: **A-C**, 5 mm; **D-H**, 500 µm.

Technovit® 7100 is used as mounting medium of biological objects for histological sectioning but requires the plasticizer poly(ethylene glycol) 400 to obtain serial sections (Gerrits & Smid 1983). Technovit® 7100 cannot be dissolved by organic solvents again once hardened (Vermathen 1993). This limits the number of possible stains and requires that the sections are also mounted in Technovit® 7100 after staining; however, the topography of the cells

may be preserved better, and thinner histological sections can be obtained leading to improved resolution (Vermathen 1993). Whereas uncured Entellan®, Eukitt™, Elvacite® 2044, and Elvacite® 2046 are soluble in organic solvents only, HPMA is partly miscible with water (6–10%). During exposure to simulated sunlight, scissioning reactions of the polymer chains predominate in lower methacrylates like poly(ethyl methacrylate), but the polymer chains cross-link in the higher methacrylates (Horie 2011, p. 153). Cross-linking results in reduced or total insolubility in organic solvents, as has been demonstrated for poly(*n*-butyl methacrylate) (Horie 2011, p. 159). "It is possible to construct copolymers with the required physical properties and with the desired balance of scissioning/cross-linking behaviour" (Horie 2011, p. 155). Poly(hydroxypropyl methacrylate) (= HPMA) has been shown to represent a health risk for humans (Taylor 1989). It should be assumed that other polymethacrylates are quite hazardous for humans too.

Phytoplankton may be mounted as bulk preparations on a glass slide in LR White resin, made by London Resin Company (Linskens 1996). Unpolymerized LR White is miscible with up to 12% of water by volume (Newman 1987). The medium seems to consist mainly of poly(hydroxy di-ethoxylated bisphenol A dimethacrylate) and the catalyst dibenzoyl peroxide (Available from: http://www.agarscientific.com/media/import/AGR1281_LR_White_Resin_medium_catalysed_MSDS.pdf, accessed 01 July 2016; Newman 1987). Specimens may be cut out of the flat resin layer with a scalpel. The resin has to be cured in an oven at 50°C for 48 hours under the exclusion of oxygen, e.g., sealed by Thermanox™ coverslips in a nitrogen-rich atmosphere (Linskens 1996) or in a gelatin capsule, in order to prevent a tacky surface due to unpolymerized resin (Sanderson 1994, p. 71). However, polymerized LR White remains hydrophilic and is, therefore, swelled by moisture; the amount of cross-linking and, therefore, of swelling depends on the curing conditions (Newman 1987). Specimens are also well suited for TEM and because of its hydrophily for antibody labelling after fixation in glutardialdehyde and picric acid (Newman *et al.* 1982).

3.7.17 Polyester

Polyesters like Bio-Plastic® (= Polylite®) used for casting macroscopic biological specimens have also been suggested as mounting medium for microscope slides (Tab. 5; Senior 1970). In the case of Bio-Plastic® like in most other polyesters, the unsaturated polyester pre-polymers react with styrene, catalyzed by methyl ethyl ketone peroxide (Tab. 5; Horie 2011, p. 283). However, not all unsaturated groups of the polyester pre-polymer react and, therefore, represent a "source of instability", which are prone to attack by oxygen and water; exposure to light leads to yellowing of polyesters (Horie 2011, p. 285). One case of a macroscopic specimen is known, where the resin turned milky and opaque after about 22 years (Meurgues 1982).

3.7.18 Poly(vinyl acetate) copolymers

The synthetic mounting medium Vinylite seems to be composed at least of the copolymer poly(vinyl chloride-co-vinyl acetate), a Flexol plasticizer, and the solvent Cellosolve™ (Fig. 12; Skiles & Georgi 1937; Gray & Wess 1951; Lillie *et al.* 1953). The name Vinylite is no longer current (Horie 2011, tab. 7.1). Stains fade rapidly within one or two weeks except methenamine silver, which lasts for months (Lillie *et al.* 1953, p. 71).

For plant material except algae, Metcalfe & Richardson (1949) mixed Solvar S.357, a copolymer of 30–37% poly(vinyl acetate) and poly(vinyl alcohol), with water and formaldehyde or thymol to prevent potential growth of fungi; alternatively, lactophenol was included in the medium. Slides may last at least 18 months, but crystals growing radially from the specimen may develop after one year in the medium containing lactophenol (Metcalfe & Richardson 1949). Specimens can be stained with cotton blue but not with hematoxylin and safranin, because the stains tend to migrate into the mounting medium.

Poly(vinyl acetate) like Mowilith (Hoechst, no longer available under this name according to Horie 2011, p.138) is transparent, but shrinks considerably on drying on a slide (Perruche 1933). Poly(vinyl acetate) is "usually slightly branched" and swollen by water (Horie 2011, p. 137). The cured resin seems to remain flexible at room temperature, but shows cold-flow behavior and picks up dirt easily. UV light causes oxidation and cross-linking (Horie 2011. p. 137).

3.7.19 Poly(vinyl alcohol)

General remarks. Poly(vinyl alcohol) has been introduced both as a mounting medium and for dehydrating histological samples by Lubkin & Carsten (1942, 1944; Fig. 12; Tab. 1). It has been used as the polymer of home-made media for mounting arthropods and contains glycerol and lactic acid; acetone, chloral hydrate, or phenol were included or not; the medium is also commercially available (Tabs 5, 12, 13; Downs 1943; Salmon 1947, 1951a, 1951b, 1954; Gray & Wess 1950; Brown 1951; Heinze 1952; Lipovsky 1953; Rutledge 1954; Russel 1961; Singer 1967; Heikinheimo 1988; Koomen & Vaupel Klein 1995). Zander (2014) suggested a home-made mounting medium, Elmer's Washable Clear School Glue™, or Colorations™ Washable Clear Glue for delicate plant specimens; all media seem to consist mainly of a poly(vinyl alcohol), glycerol, and water. Both home-made media and the glues by Elmer's and Coronations are supposed to be re-mountable by soaking, but no indication is given about how long after mounting the polymer remains dissolvable (Zander 2014). Ribeiro (1962) mixed a poly(vinyl alcohol) with chloral hydrate, formic acid, and phenol. In his later recipe, he omits both chloral hydrate and phenol (Ribeiro 1967). Glycerol, and more specifically, the water bound by the hygroscopic glycerol acts a plasticizer (Spurr 1954; Witte 1976). Poly(vinyl alcohols) are produced in different qualities. Two numbers usually indicate the degree of polymerization (first number) and the percentage of saponification (second number). Unfortunately, this detailed information is not always provided in the literature (Downs 1943; Gray & Wess 1950; Brown 1951; Ribeiro 1962).

Spurr (1954) included in his recipe 40% water, 18% Elvanol 51-05 (DuPont, low viscosity grade), 34% cadmium iodide, and 8% fructose, which acted as plasticizer. The refractive index of the dry medium varies from $nD^{20°C}$ = 1.5150–1.6020 depending on the amount of cadmium iodide. Cadmium iodide prevents bleeding of many stains in the aqueous medium for at least 9 months. However, crystals develop in the medium or tissue if sections are stained with acridine yellow, indulin scarlet, new methylene blue N, or pyronin Y (Spurr 1954). Fading of stains such as hematoxylin can be prevented by reducing the amount of cadmium iodide to about 6%.

Salmon (1947, 1951a, 1951b) experimented with Elvanol Type A (51.A.05) and Type B (70.A.05) as well as with Elvanol 71–24 from Du Pont, with a W.28-02 from an unknown company (Wacker?), and a No. 52-22 and No. 72-51 from ICI. The latter two poly(vinyl alcohols) seem to develop crystals within a short time (Salmon 1951b). Lubkin & Carsten (1942, 1944) selected RH-393 from E.I. du Pont de Nemours. Downs (1943) and Lipovsky (1953) chose the alcohol from E.I. du Pont de Nemours (RH-349), which dissolved in cold but not in warm water. Heinze (1952) used poly(vinyl alcohol) either from Wacker (W 28-02) or from Bayer (S 70). Beer (1954, p. 1111) recommended Type B low-viscosity Elvanol 70-05 and 90-25.

Danielsson (1985, p. 384) suggested for his medium Polyviol, a mixture of ethanol, water, lactic acid, and two different poly(vinyl alcohols) "with a high difference in the degree of polymerization and both must have a high percentage (98%) of saponification", viz, Mowiol® N 4-98 and N 56-98. This recipe was also followed by Heikinheimo (1988). The idea goes back to a recipe for Polyviol 17 by Frej Ossiannilsson who mixed Mowiol® N 30-98 and N 90-98; production of these latter alcohols by Hoechst was discontinued (Danielsson 1985). Collembola mounted in poly(vinyl alcohol) usually shrink if treated with lactic acid or lactophenol before mounting (Rusek 1975). Cavities and crystals may develop over time (Fig. 19E, F).

Polyvinyl lactophenol. Gas bubbles in the medium on the microscope slide may be removed by dropping small droplets of 70% ethanol from a dissecting needle onto the medium; gas bubbles will migrate to the surface of the medium and burst (Koomen & Vaupel Klein 1995, p. 435).

Polyvinyl lactophenol causes alkaline stains to fade because of the lactic acid in the medium (Salmon 1951a). In addition, granular precipitations occur, but usually slides last longer than four years (Salmon 1951a). Droplet-like structures are supposed to represent a segregation of the chemical components of the medium (Fig. 7F) originating from an unsuitable intermedium during the mounting process (Woelke & Göke 1984, figs 1, 3). These authors also suggest that radially growing crystals or other crystals develop if the medium dries too quickly or at a too high temperature (beyond 40°C: Russel 1961) if the storage temperature of the slides is too low for some time, or if the mounting medium contains chloral hydrate (Woelke & Göke 1984, pp. 212–213, fig. 2). In addition, a coverslip seal is necessary to prevent the evaporation of water and consequently shrinkage of the medium and uptake of humidity from the air; slides may last at least 30 years (Hirschmann & Woelke 1960; Woelke & Göke 1984, p. 211; Hooper 1986b). If the medium sets in an unsealed slide, a specimen may be crushed by the coverslip within two years (Müller 1983), probably because of evaporation of water. Crystals and turbidity are observed in

some slides (Bink 1979). The mounting medium turns brown and thickens (polymerization?) after exposure to light for several months (Lipovsky 1953). Huys & Boxshall (1991, p. 451) report too intense maceration of copepods within 10 years, formation of crystals, and drying out. Cuticle and setation of marine mites will be overly macerated within a few years (Bartsch 1988). Hirschmann (1984) stated that slides might last at least 25 years. Phenol in the media seems to have caused shriveling of delicate specimens, so this chemical is omitted in a later formula (comp. Salmon 1951a and 1954). Mounts of Kinorhyncha mounted in polyvinyl lactophenol exhibit numerous cavities and at least four different kinds of crystals, namely irregularly growing crystals, aggregations of spherical crystals, radially growing crystals, and crystals with a rectangular basic structure (Figs 18B–H, 19A–D; Neuhaus 2013, p. 278, fig. 5.5.1.C–D, 5.5.2.A–B). Salmon (1947, 1951a) attributed the development of precipitations in the medium to unbalanced proportions of poly(vinyl alcohol), lactophenol, and water. Lipovsky (1953) stressed that phenol plus lactic acid must not take up more than 50% of the medium by volume to avoid a tacky and soft medium (see also Salmon 1947, 1951a); lack of these components would cause problems with re-mounting a specimen. Danielsson (1985) suggested, on the contrary, that chloral hydrate and phenol were responsible for the loss of volume during drying of the mount and formation of cavities from the periphery of the coverslip and omitted these substances. An incorrect proportion of poly(vinyl alcohol) in the mounting medium is supposed to crack the coverslip over the specimen (Lipovsky 1953). The permeability to oxygen is quite low but increases with the uptake of water (Mills & White 1999, p. 132; Comyn 1986, p. 284). Generally, poly(vinyl alcohols) shrink by about 50% on drying (Heikinheimo 1988), are hygroscopic (especially at relative humidity > 75%) and light stable; they may cross-link over time releasing water but remaining water-soluble (Witte 1976; Mills & White 1999, p. 132; Horie 2011, p. 143). If glycerol, lactic acid, and phenol are included in the medium, the latter seems to become insoluble in water (Brown 1951). However, conflicting evidence is given that re-mounting seems to be possible by soaking for one to several days either in water (Tab. 13: Technique C; Salmon 1954) or in 70% ethanol (Koomen & Vaupel Klein 1995, p. 435) or not possible at all (Ossiannilsson 1958; Singer 1967).

Fluoromount G™. The commercial Fluoromount G™ was (Sørensen & Pardos 2008, p. 35), and still is, much favored for mounting Kinorhyncha, but turned out to segregate into its chemical components in the form of droplets (Fig. 20D) and to develop cavities and crystals under the coverslip over time (Fig. 20A–C, E, F; Neuhaus 2013, pp. 278, 308, fig. 5.1.18B–D, 2017, fig. 1A–C). Specimens of *Tubulideres seminoli* Sørensen *et al.*, 2007 and *Wollunquaderes majkenae* Sørensen & Thormar, 2010 were originally mounted in Fluoromount G™ and sealed with Glyceel sometime after 2003 and after 2006, respectively. Of the 25 slides, the mounting medium of 10 slides (= 40%) revealed by the year 2013 smaller or larger, usually circular cavities anywhere under the coverslip now in almost all cases affecting microscopic investigation of the specimens (Fig. 20B, C). In addition, three kinds of crystals occurred on all slides, anywhere under the coverslip and even on the specimens (Fig. 20C, E, F). Of the 12 specimens of *Polacanthoderes martinezi* Sørensen, 2008 mounted around 2005 in Fluoromount G™, 11 (= 92%) showed cavities in the year 2015 and all 12 (= 100%) crystals. The most frequent type of crystals found in these three species on 31 slides (= 84%) appeared as very small, flat, platelet-like discs (Fig. 20A; Neuhaus 2013, fig. 5.1.18B–D), a second type of circular crystals on 9 slides (= 24%) seemed to grow radially from a center (Fig. 20B–E), and a third type of crystals exhibiting an almost rectangular growth pattern could be discerned on five slides (Fig. 20B–D). The latter two types of crystals were especially disastrous, because they often formed at the specimen and may become quite large, finally overgrowing the specimen (for comparison see Fig. 20E, F; Neuhaus 2017, fig. 1A–C). Usually, only one type of crystal occurred on a given slide, but occasionally a slide yielded several types of crystals. In summary, of 37 slides of *P. martinezi*, *T. seminoli*, and *W. majkenae* 19% were heavily impacted by crystals at and on the specimen. In 18 slides (= 49%) the specimen was surrounded by a large cavity and in 8 slides also by extensive crystals; these 18 slides will be unusable for scientific studies unless re-mounted soon. At least, Fluoromount G™ is soluble in water even after about 10 years (Tabs 6, 13; Neuhaus pers. obs.).

3.7.20 Silicone rubber

A silicone rubber, viz, Loctite® Hybrid Glue containing (3-(2-aminoethyl) aminopropyl) trimethoxysilane, has been suggested as a permanent mounting medium for type material of Protozoa in museum collections; slides last at least one year (Fig. 12; Criado-Fornelio *et al.* 2014). Considering the problems with silicone rubbers addressed

below in chapter 3.8.14 Silicone rubber, this medium may not really represent a long-lasting alternative to Canada balsam as claimed by the authors.

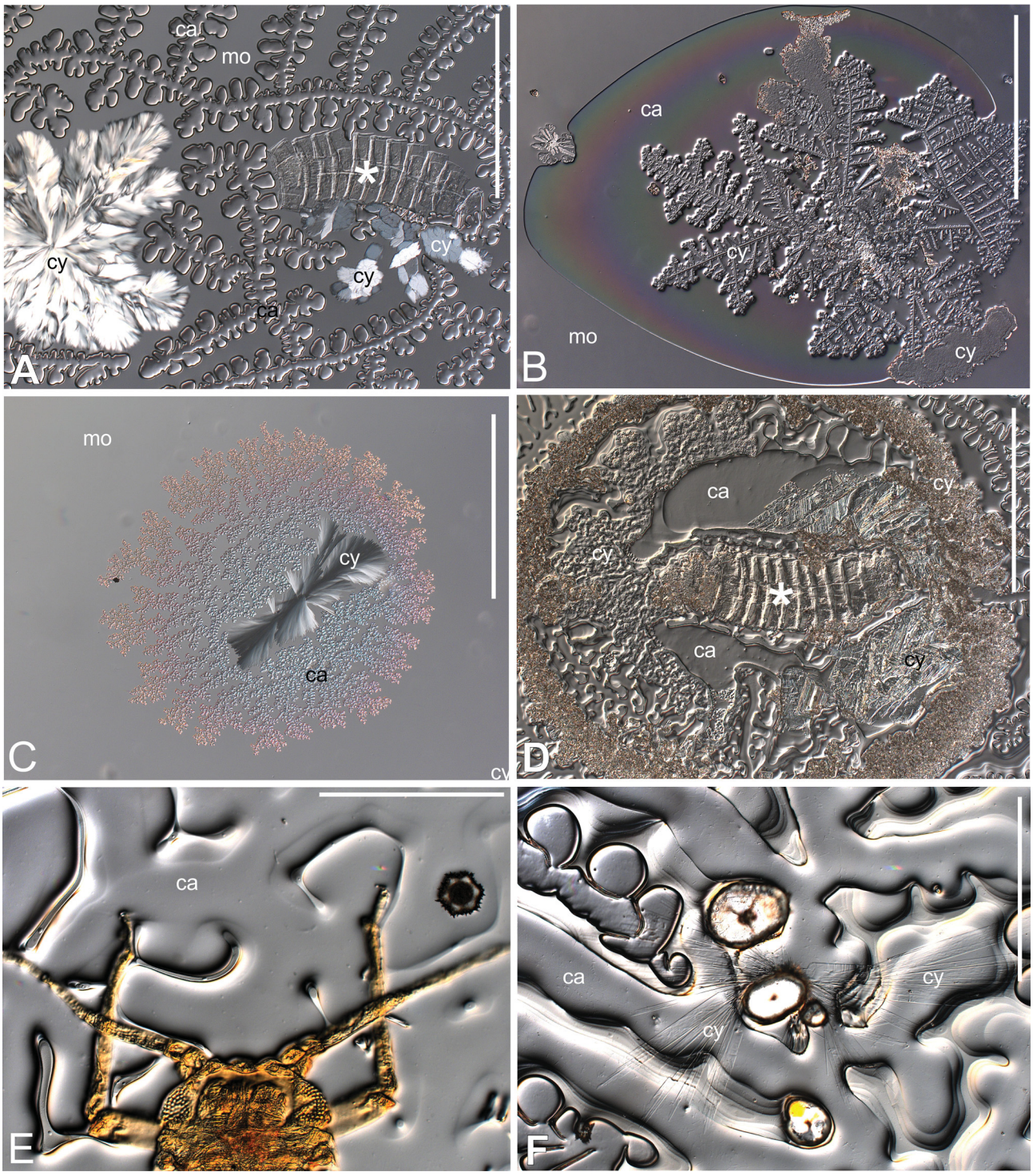


FIGURE 19. A-D. Kinorhyncha mounted in polyvinyl lactophenol between 1985 and 1990 by Neuhaus, unringed; currently not affected mounting medium (mo) and areas at different stages of deterioration with extensive cavities (ca) and formation of different kinds of crystals (cy). Specimens marked by asterisk. **E, F.** Aphidina mounted in Polyviol; now deteriorating with cavities and crystals. **A-F**: DIC. Scalebars: **A-D**, 500 µm.

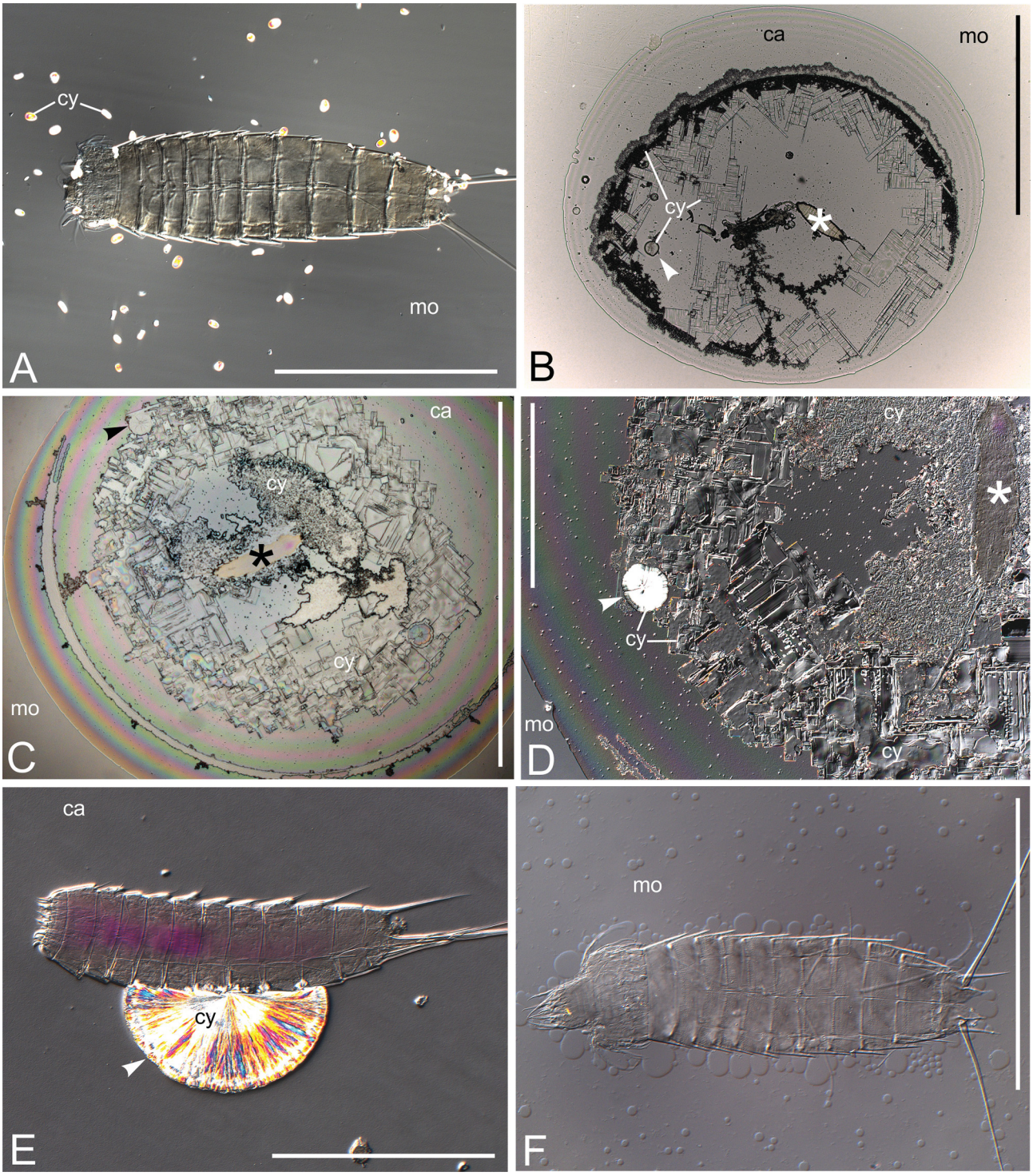


FIGURE 20. A-F. Kinorhyncha mounted in Fluoromount G™ between 2002 and 2008 (**A**, **B**), 2003 and 2007 (**C**, **D**: same slide), and 2006 and 2010 (**E**) by Sørensen (**A-E**) and 1999 and 2012 by Herranz (**F**). Currently not affected mounting medium (mo) with severe deterioration like cavities (ca), platelet-like (**A**), rectangularly growing (**B-D**), radially growing (**B-E**, arrowheads), small chaotic (**D**) crystals (cy), and segregation of ingredients of mounting medium resulting in formation of bubbles (**F**). Specimens marked by asterisks. **A**, **DF**: DIC; **B, C**: bright field illumination. Scalebars: **A**, **E**, 300 µm; **B, C**, 2 mm; **D**, 500 µm; **F**, 200 µm.

3.7.21 Visikol™

The formula of Visikol™ is proprietary (http://www.visikol.com), but glycerol, a preservative, and a polychlorinated alcohol are mentioned as being present (Villani *et al.* 2013; Tab. 1). A patent by the same authors states that the medium may consist of glycerol, trichloroethanol, trichloroacetic acid, one or more C1-C6 alcohols, one or more inorganic (hypochloric / phosphorous / sulfuric acid) and organic acids (acetic / citric / formic / lactic acid), a synthetic resin like poly(vinyl pyrrolidone), and additional compounds like a buffering system and preservative (Simon *et al.* 2015). From this patent, it becomes obvious that Visikol™ represents a mounting medium that macerates a specimen chemically at the same time. Long-time experience with Visikol™ is lacking as yet. However, based on the experience documented in the literature (Tabs 5, 6) with synthetic mounting media containing macerating agents, it may be expected that Visikol™ will show similar problems in durability as the longer known mounting media.

3.7.22 Water-glass-Glycerol

A mixture of water-glass (= sodium silicate; Fig. 12) and glycerol has been suggested in different proportions as mounting medium. In the recipe with a high proportion of water-glass (Creaser & Clench 1923), the glycerol functions as a plasticizer "to keep the water-glass from crystallizing"; in the method with a lower proportion of water-glass, the latter is used as a kind of coverslip seal "to hold the glycerin to the microscope slide" (Zander 2014). With the evaporation of water from the medium in the latter recipe, water-glass migrates to the periphery of the coverslip and precipitates as whitish or brownish crystals, and the central area of the mount with the specimens contains mainly glycerol (Zander 2014, p. 3). Mounts should be ringed (Creaser & Clench 1923). Whereas the latter authors report that material mounted in plain water-glass would suffer from crystallization of the medium within three months, Quisumbing (1931) suggested just this and claims that mounts would last at least one year.

3.7.23 Zeiss W15, L15, and L25

In search of a mounting medium with a satisfying refractive index of nD = 1.515 for investigation of sections with phase contrast, von Hirsch experimented with several mounting media and finally developed a medium composed of an ester of phthalic anhydride and glycerol (Hirsch & Hager 1955, p. 152). With an increasing amount of phthalic anhydride during the synthesys of the medium, the refractive index also increased. A combination of glycerol:phthalic anhydride 2:1 resulted in an index nD = 1.515 (Hirsch & Hager 1955, p. 152). Subsequently, the company Carl Zeiss in West Germany offered several mounting media based on the recipe of von Hirsch, *viz*, P 50, Phako 515, and Phako 525 especially designed for phase contrast microscopy (Zölffel, pers. com.). These media were later renamed W15, L15, and L25, respectively, and contained phthalic anhydride, glycerol, and water (water = Wasser: W15) or an organic solvent like ethanol (solvent = Lösungsmittel: L15, L25) and possibly other, proprietary ingredients (Fig. 14; Tab. 5; Zölffel, pers. com). Whereas W15 remained liquid, L15 and L25 hardened over time. The mounting media can still be ordered from Carl Zeiss Microscopy and are prepared on request. Written information about W15, L15, and L25 is very limited. Several authors suggest to use W15 (Westheide & Purschke 1988; Huys & Boxshall 1991). A brochure by Zeiss shows an image of the media (Anonymous 1960). The brochure also contained an inlay with additional information about how to apply the media (Zölffel, pers. com.).

3.7.24 Media with a high refractive index (Aroclor®, Coumarone, Hyrax, Naphrax™, Novolacs, Pleurax, Styrax)

General remarks. Mounting media with a high refractive index are mainly used in biology especially for the study of diatoms but also for marine mites and Radiolaria (e.g., Newell 1947; Hasle & Fryxell 1970; Göke 1973, 2000; Bartsch 1988; McLaughlin 2012). The diatom shells possess a refractive index of nD = 1.43, so any medium must

reveal a considerably higher refractive index in order not to mask fine details (Beck 1959). A historical overview of such media and how some of the media may be created was provided by Van Heurck (1898), Beck (1959), and Meller (1985). Although the articles by Beck and Meller were published in a journal addressing as one key group German-speaking amateurs, the recipes contained quite hazardous components and, therefore, certainly required considerable understanding of the underlying chemical processes and laboratory facilities including a hood. With current knowledge, many recipes found in these and other references have to be regarded as quite adventurous and represent a serious risk for human health and the environment, e.g., media containing arsenic sulfide (= realgar), arsenic bromide, and triphenyl arsenic (Meller 1985). Diatom shells have also been sputter-coated with realgar or arsenic selenide and observed mounted in balsam and Hyrax (Hanna & Grant 1940; Spence 1941a). Transmission of Pleurax for UV light is very limited, and Naphrax and Styrax reveal significant fluorescence, so observation of a specimen in these media is only possible with white light but not with UV light (Höbel 2008). Summarizing information about mounting media with a high refractive index is also found in McCrone (1984), Loveland & Centifano (1986), and McLaughlin (2012).

Several media like Alcarin, Aroclors®, Clearax (G.T. Gurr Ltd.), Diatopan (Chroma), Mikrops (Flatters and Garnett), and Plexisol (Kosmos Lehrmittel) are no longer available, because the manufacturer does not exist anymore or because the production of certain components of the media were forbidden (Beck 1959; Meller 1985; Göke 2000; Wiggins & Drummond 2007) and are not treated in more details here except for Aroclors®. The same is true for some media identified simply by numbers but without much information about their origin or the ingredients they contain (Liva 1983). Clearax and the Yellow Medium after Smith form crystals over time (Meller 1985; Göke 2000). Rosenfeldt (1984a, 1984b, 1984c) provides information on how to check the refractive index of mounting media with two cheap methods and to calculate the refractive index of chemicals based on physical data of their atoms. The basic idea of this author is that amateurs should develop a mounting medium with a high refractive index, because a company will not do so given the small number of users for such a product.

Aroclors®. Aroclors®, containing polychlorinated biphenyls and polyphenyls in variable composition, were traded in different countries under different names "such as Askarels & Aroclors (USA), Phenochlor & Pyrolene (France), Clophen (Germany), and Kanechlor (Japan). The last two digits of the commercial name indicate the percentage of chlorine in each mixture. E.g. Aroclor 1242 contains 42% by weight of chlorine." (Smith 2003, p. 44). Aroclors® have been suggested in searches of mounting media with a high refractive index and applied for decades by, e.g., mineralogists, diatom and Radiolaria specialists, and microscopists in forensic and conservation sciences (Tab. 1; Frison 1955; Hasle & Fryxell 1970; Göke 1973, 2000; McCrone 1984), but these would certainly not be used nowadays because of their toxicity, and their production has ceased (Göke 2000). Aroclor® 5442 is a polychloroterphenyl; the medium may form crystals over time, which disappear upon heating (McCrone 1984). Aroclor® 1262 and Aroclor® 4465 are viscous at room temperature (Loveland & Centifanto 1986, p. 231). The latter can be diluted in xylene 1:1 and is miscible with up to 40% other resins like dammar resin in order to adapt to a range of refractive indices from nD = 1.52–1.666, which seems to be important for the study of Radiolaria; the mixture is heated to 100–120°C during preparation of diatoms on microscope slides (Göke 1973, 2000). Aroclor® 1254, Aroclor® 1260, and Aroclor® 5442 have also been used for mounting diatoms; Aroclor® 5460 alone becomes brittle but is supposed to make permanent microscope slide mounts if used in a mixture with Styrax (Brigger 1960; McCrone 1984, p. 280; Loveland & Centifanto 1986, p. 231; McLaughlin 2012, pp. 123–124). Aroclor® 1242 has been suggested as a plasticizer for poly(*iso*-butyl methacrylate) (Loveland & Centifanto 1986, pp. 191, 231).

Coumarone. Coumarone was probably introduced as a mounting medium in 1933 by Perruche (Tab. 1); it is made from naphthalene, which yellows slightly over time (Perruche 1933; Frison 1952a, 1952b). The resin must contain bromonaphthalene or a plasticizer like castor oil in order not to become too brittle and the coverslip detaching; it is dissolved in benzene, toluene, or xylene (Perruche 1933; Frison 1952a, 1952b, 1955). Frison (1955, p. 208) suggested a mixture of Aroclor® 1262 and Coumarone in equal parts plus 1% castor oil as plasticizer.

Hyrax. Hyrax was synthesized from aniline, formaldehyde, and sulfur and, therefore, named originally A.F.S. (Tab. 1; Hanna 1927, 1930). The medium is soluble in benzene, toluene, and xylene (McLaughlin 2012, p. 117). It becomes very brittle on drying, so the coverslip may detach easily (Göke 1973, p. 278, 1984, 2000, p. 373). Hyrax may grow turbid and may blacken over time (Loveland & Centifanto 1986, p. 196). The medium does not seem to be available anymore.

Naphrax™. Naphrax™ (Tabs 1, 5) belongs to the better known mounting media with a high refractive index

suitable especially for the study of diatoms and is still available at least in the USA, Great Britain (Brunel Microscopes Ltd.), and Germany (Biologie-Bedarf Thorns), both in a version with and without toluene. A detailed instruction of how to mount diatoms in Naphrax™ is given by Webb (1997) and Acker *et al.* (2002). The original recipe by Fleming (1943, 1954) was patented by Fleming (Wicks *et al.* 1946) and requires extensive laboratory work, which is also true for a modified synthesis posted on a site for amateurs (Tab. 5; G. Rosenfeldt: Hochbrechende Einschlußmittel (PLEURAX / NAPHRAX / ZRAX), Available from: http://www.mikrohamburg.de/Tips/T_Hochbrechende%20Einschlussmittel.html, accessed 14 July 2015). The chemical nature of the final polymer has not been elucidated, so only the source ingredients of this mounting medium are mentioned in Table 5. Naphrax™ reveals darkening after about 50 years (Brown 1997, fig. 25). Naphrax™ becomes very brittle on drying, so the coverslip may detach easily (Göke 1973, p. 278). Also, crystallization occurs over time (Göke 1984). Moisture is supposed to cause cloudiness of the medium and precipitations (McLaughlin 2012, p. 120).

Novolacs. Novolacs are phenol-formaldehyde resins (PF resins) like Bakelite and have been used for the preparations of fresh-water algae and microcrustaceans, e.g., BRPB 5215 soluble in 60–100% ethanol from Union Carbide (now The Dow Chemical Company). The resin has to be further cleaned chemically and physically in the lab in order to get rid of excess phenol and other contaminants (Crumpton & Wetzel 1980, 1981). Specimens are mounted from ethanol and survive at least one year. BRPB 5215 does not seem to be manufactured anymore, at least not under this name. Perruche (1933) stated that Bakelite A quickly changed colored specimens on a microscope slide. Horie (2011, p. 297) mentioned that phenol-formaldehyde resins were brittle and hard, which may suggest that they are not suitable for long time storage.

Pleurax. Pleurax was initially, and still is, created from sulfur, phenol, and anhydrous sodium sulfide for about 8 hours (Tab. 1; Hanna 1949; Hepworth 1994). Since anhydrous sodium sulfide was unavailable to Stosch (1974), he used anhydrous sodium carbonate instead. The latter acts as a catalyst. The preparation of the medium requires *inter alia* heating at 180–190°C for 5–9 hours. The polymer consists of phenol rings connected via sulfur bridges. In principle, the sulfur may oxidize to sulfur dioxide causing cloudiness, and the phenolic hydroxide may form chinones, which darken the medium (Rosenfeldt, see above). The resin is dissolved in acetone, ethanol, or isopropyl alcohol (Hanna 1949; Beck 1959; Stosch 1974; Hepworth 1994). It remains open whether or not cloudiness occurs in aged slides. However, slides do turn yellow or brownish over time (Hepworth 1994). Pleurax seems to last at least 16 years (Hanna 1949) and is Available from: Chemlab in Germany. It becomes brittle over time (Göke 1984).

Styrax. Styrax resin (Fig. 9; Tab. 5) has been introduced as mounting medium for diatoms in 1883 by Van Heurck (Tab. 1; Bracegirdle 1978, p. 92). It can be prepared from the raw resin and, in this case, requires removal of particulate and aqueous matters in a time-consuming procedure, before it can be used in microscopy (Van Heurck 1898; Needham 1924; Beck 1959; McLaughlin 2012, p. 119). A commercial version seems also to be or to have been available. The latter two authors report cloudiness and granular precipitations in the medium if Styrax is not cleaned properly. Needham (1924) regarded the precipitations as benzoic and cinnamic acid. The same situation is also suggested for balsam of tolu (Needham 1924). The medium dries over a long period of time and turns yellow on heating, which is necessary for mounting diatoms (Göke 2000). If Styrax is heated too quickly and too hot, the medium is responsive to mechanical shock and will separate from glass surfaces (McLaughlin 2012, p. 138).

3.7.25 Fungi

Fungi have been claimed to represent a threat to slide collections especially under humid conditions (Heinze 1952; Brown 1997: p. 6; Gütebier 2011; Zander 2014), but we have found no convincing evidence. Possibly, such evidence may have been presented in certain taxonomic articles. Gütebier's photographs (2011, p. 75, figs 18, 19) do not document hyphae of fungi but bar-like remnants of the mounting medium, which has partly withdrawn from the space between slide and coverslip; this phenomenon has also been found in other media and is not related to fungi in any way (Neuhaus pers. obs.). One may be misled by a photograph of Lillo *et al.* (2010, fig. 3, p. 287) showing an aphid invaded by hyphae; however, the text clearly states that the host was infested during improper storage before (!) mounting. However, microorganisms including fungi are able to feed on picture varnishes made

from natural plant resins or on synthetic resins used in conservation (e. g., Cappitelli *et al*. 2004; Romero-Noguera *et al.* 2008, 2010; Sterflinger 2010). Fungi and other microbes may even grow on clean surfaces of glass including borosilicate glass and corrode the surface by delivering acidic, alkaline, and chelating agents (e. g., Kerner-Gang 1977; Drewello & Weissmann 1997). To our knowledge, evidence for a severe threat of microscope slide collections by fungi has not been documented yet.

3.7.26 Discoloration

Discoloration of mounting media is supposed to originate from "residual preservatives such as phenolics and formaldehyde" in specimens, so thorough washing both after fixation and after maceration or clearing and before mounting is recommended (M. Ipe and B. Pitkin in Disney & Henshaw 1988; also Gardner 1975 for plant material). In addition, phenol in Canada balsam (= phenol balsam) as well as macerating agents like chloral hydrate and clearing agents like clove oil have been held responsible for blackening (Wagstaffe & Fidler 1955, p. 173; Quednau in Eastop 1985, p. 270; Stroyan in Eastop 1985, pp. 269–270; Brown 1997; Brown & Boise 2005, p. 26, 2006, p. 16). Deterioration of phenol balsam starts in the periphery of the coverslip indicating that oxygen may be involved (see chapter 3.8.1 General aspects of coverslip seals; Essig 1948; Loveland & Centifanto 1986, p. 184; Brown 1997, p. 8). A mixture of phenol, ethanol and Canada balsam darkens within three weeks (Wirth & Marston 1968). However, Quednau (in Eastop 1985, p. 270) regarded chloral hydrate as the cause of blackening. A blackened specimen may be bleached by rinsing in strong alcohol followed by bleaching in 25% ammonia and 30% hydrogen peroxide (Brown & Boise 2005, p. 26, 2006, p. 16).

Deterioration of gum-chloral media and finally of the specimen may start at the specimen with pink or bluish areas gradually turning black; the cuticle of insects becomes more and more transparent until invisible (Stroyan in Eastop 1985, pp. 269–270; Brown 1997, p. 9; Brown & Boise 2005, 2006). Once blackened, the insect cuticle is irreversibly damaged and cannot be restored, even if the specimen is re-mounted (Brown 1997, p. 10). The blackening is suggested to originate from insufficient washing of the macerating and clearing agents like potassium hydrate, chloral hydrate, and phenol; exposure to light and high temperature may add to the problem, and phenol probably represents the most problematic substance in gum-chloral media of insect collections at the Natural History Museum in London (Brown 1997, pp. 9–10; Brown & Boise 2005, p. 27, 2006, p. 17). Blackening starting from the periphery of the coverslip has been attributed to a reaction of the gum-chloral medium with Euparal or Murrayite used for ringing (Disney & Henshaw 1988; Brown 1997, pp. 9–11; Brown & Boise 2005, p. 27, 2006, p. 17). Un-ringed slides with Hoyer's medium containing no phenol may turn dark brown within less than 20 years, probably because of the iodine and potassium iodide included in this specific case (Neuhaus pers. obs.).

3.8 Coverslip seals

3.8.1 General aspects of coverslip seals

Coverslip-slide mounts are usually sealed if the mounting medium is liquid (e.g., containing formaldehyde, glycerol, or lactic acid) or consists of water-based media such as gum-chloral media (Adam & Czihak 1964; Brown 1997). Resins like Canada balsam do not require initial ringing, because the solvent must be able to evaporate so the mountant can harden (Spence 1939; Wagstaffe & Fidler 1955b, p. 197; Brown 1997). Ringing of such slides may be useful at a later time in order to provide some protection and to slow down discoloration and evaporation of less volatile substances of the resin, so the medium does not become brittle (Wagstaffe & Fidler 1955, p. 174; Brunner & Blueford 1986). Generally, a good seal should

(1) stabilize the mount physically especially for studies of glycerol, glycerol-gelatin, and glycerol-paraffin slides with oil immersion objectives,
(2) prevent uptake and loss of water in aqueous mounting media (Travis 1968; Brown 1997, 1998),
(3) prevent or at least retard diffusion of oxygen into the mounting medium thus discoloring stained sections (Loveland & Centifanto 1986; Brown 1997, 1998),
(4) remain flexible over decades,
(5) not react with the mounting medium, and

(6) be removable easily if a specimen has to be re-mounted. The latter includes knowledge of the ingredients of the sealant in order to apply the appropriate conservation method.

The approach mentioned above takes into consideration general concepts in conservation science (Shashoua 2008; Horie 2011, pp. 3–13).

"When the ring fails in a dry environment, evaporation, shrinkage, discoloration and cracking [of the mounting medium, the authors] can occur. When relative humidity and temperature are too high, sweating can occur making a sticky slide and which promotes fungal attack of the mountant especially in water based mountants." (Brown 1998, p. 44). Ternant (1935) attributed the failure of a coverslip seal for liquid mounts to the lack of attachment of the seal to the glass surfaces. Consequently, he suggested grinding both the glass slide and the coverslip in order to provide a rough surface attachment area and recommended a spacer plus a ring of paraffin or goldsize below the extensive coverslip seal (Ternant 1935, fig. 1). Cushing (2011) reported problems with coverslip seals for pollen mounted in silicone oil. Zander (1997) suggested letting glycerol permanently evaporate from an open container in a closed cabinet in order to reduce evaporation of glycerol from slides.

Unfortunately, experiments about the properties of coverslip seals are rare. Allington & Sherlock (2007a, 2007b) studied to what extent a seal could act as a barrier to oxygen diffusing into the mounting medium by exposing mounts alternatingly to room temperature and freezing at -20°C to -40°C for 7 cycles. With this thermally-induced mechanical stress, of 13 sealants only Canada balsam retained a permeability barrier against oxygen and kept its chemical and physical integrity. It seems that the aforementioned experiment ran for less than a month (Allington & Sherlock 2007a, 2007b). It remains open as to whether or not the results of the experiment can be applied to real slide collections stored for decades and hopefully centuries (see also chapter 3.7.2 Permeability of polymers for gases and vapors). The senior author's own observations of microscope slides with Aphidina mounted in Celochloral or Canada balsam reveal that the medium in the center of such slides may still show "normal" coloration, whereas the peripheral medium suffers from discoloration and a yellowish mounting medium (Fig. 15E, F). This suggests that the mounting medium could not provide a diffusion barrier against oxygen. It cannot be expected that a coverslip seal would do a better job, because the seal will be limited in thickness in order to allow study of specimens with oil immersion objectives. This also raises the question for water-based mounting media like gum-chloral media of how effectively water is kept in or out of a mount by a coverslip seal under fluctuating environmental conditions (see also chapter 3.7.2 Permeability of polymers for gases and vapors and chapter 3.7.14 Gum-chloral media; Tab. 7).

Several principal factors impacting the longevity of coverslip seals can be hypothesized:

(1) Proper construction of the coverslip seal to begin with. In order to give a good seal, the surplus mounting medium has to be removed from the slide, the glass surfaces must be totally clean for proper adhesion of the seal to the glass surface, and the sealing liquid has to be applied 2–5 times in a thin film with intermediate time for drying (Travis 1968; Henshaw 1981; Hooper 1986b; Brown 1997; Hooper *et al.* 2005). Cells for liquid media are often prepared of different coverslip seals like goldsize and asphaltum varnish, the latter applied in several layers; a colored seal may finish the seal (Rousselet 1895; Gray 1954, pp. 23–27, 31, figs 9–19; Garner & Horie 1984, p. 98).

(2) Changes in temperature throughout the seasons (Zander 1997; Allington & Sherlock 2007a, 2007b) will certainly influence the dilatation of mounting medium and seal.

(3) Mechanical stress (Brown 1997) during handling of a slide, observation with an oil immersion objective, and cleaning the coverslip from oil will impact the integrity of the seal. Both changes in temperature and mechanical stress require a considerable plasticity of the coverslip seal.

(4) The sealant may chemically react with the mounting medium (Garner & Horie 1984; Brown 1997). This is especially the case if a water-soluble mounting medium like glycerol-gelatin or a gum-chloral medium is ringed with an ethanol-containing coverslip seal like brown cement or Glyceel (Bink 1979, p. 160; Garner & Horie 1984; Neuhaus pers. obs.). Application of an intermediate layer of a different seal such as gum dammar is recommended before finally sealing with the ethanol-containing sealant (Garner & Horie 1984, p. 98).

(5) The chemical properties of the components of the coverslip seal may inherently have the potential to deteriorate over time and/or change, because the coverslip seal may react with oxygen, noxious gases (*inter*

alia from degradation of wooden cabinets), and humidity over time. A deteriorating coverslip seal will also allow bacteria and fungi to grow under favorable conditions.

(6) Generally, any coverslip seal will be permeable to oxygen, noxious gases, and humidity to some degree and, consequently, will not represent a permanent permeability barrier for decades (Tab. 11; comp. discussion in chapter 3.7.2 Permeability to gases and vapor; Schauff 1985; Gerlach *et al.* 2001; Wypych 2012). Gray (1954, p. 58) and Loveland & Centifanto (1986, p. 184) mentioned that a coverslip seal only retarded oxidation of a medium.

Various chemicals have been used alone or in various mixtures as coverslip seals for microscope slides, e.g., Araldite, asphaltum, beeswax, Bell's cement (black cellulose nitrate lacquer), brown cement, Caedax, Canada balsam, candle or paraffin wax mixed with petroleum jelly (Vaseline) or Canada balsam, caoutchouc cement, Clarite, Corseal, Euparal, gelatin, Glyceel (used in Great Britain, the North American equivalent is Zut), Glyptal (an enamel paint for waterproofing car motor parts), goldsize, gum dammar (= gum damar), gum mastic, latex paint, Murrayite, nail varnish, Permount™, poly(ethyl methacrylate) with the plasticizer butyl benzyl phthalate, polyurethane, putty (designed "for filling holes [...] in galvanized iron sheets to prevent leakage"), shellac, silicone rubber, spirit lacquer, Titebond® Glue, Venetian turpentine, water-glass, white lacquer, and white zinc lacquer (Tabs 8, 9; Behrens 1892, pp. 71–73; Mohr & Wehrle 1940; Gray 1954, pp. 651–656; Doncaster 1962; Adam & Czihak 1964; Boparai & Chhabra 1968; Travis 1968; Caveness 1969; Hooper 1970, 1986a, 1986b; Jairajpuri & Rahmani 1979; Henshaw 1981; Zander 1983; Gerakaris 1984, p. 262; Loveland & Centifanto 1986; Frahm 1990; Wilkey 1990, p. 347; Sabir 1996; Brown 1997, pp. 17–18; Hooper *et al.* 2005; Cushing 2011). Older slides are often ringed with two different media (see chapter 3.8.16 White lacquer; Behrens 1892; James 1887; Gray 1954; Garner & Horie 1984). Reports about the benefits and disadvantages of a given coverslip seal are partly disputed (Tab. 9; Brown 1997) and rarely based on experimental tests (Allington & Sherlock 2007a, 2007b). Numerous recipes of coverslip seals are found in James (1887), Behrens (1892), Gatenby & Beams (1950), Gray (1954), and Brown (1997). Gray (1954, pp. 21–41) also described in detail building of cells for fluid-mounted specimens. Table 8 lists ingredients of various coverslip seals and Table 9 their advantages and drawbacks. Below, aspects of selected coverslip seals are discussed.

3.8.2 Araldite

Araldite was suggested as coverslip seal by Boparai & Chhabra (1968). Cushing (2011) found reaction over time of the epoxy resins Epon™ 828 and 1001, Scotch™ Epoxy, and Low V™ with the silicone oil, in which pollen grains were mounted. For drawbacks of epoxy resins compare chapter 3.7.11 Epoxy resins (Araldite, Epon™, Spurr's resin).

3.8.3 Asphaltum

Asphaltum belongs to the long-known coverslip seals (James 1887, pp. 62–63; Behrens 1892, p. 71), but seems to crack on drying (Spence 1940c). The plasticizer castor oil is supposed to prevent cracking of the seal (McLaughlin 2012, p. 179). Asphaltum seems to have been applied in a mixture with linseed oil and terpentineol (Behrens 1892, p. 71). In other recipes, asphalthum has been combined with variable chemical components (Tab. 8; James 1887) and may contain a high percentage of mineral matters.

3.8.4 Canada balsam

A specimens mounted in anhydrous glycerol, glycerol-gelatin, or any other medium between a lower larger and an upper smaller circular coverslip can be sealed with Canada balsam, Caedax, Clarite, or Euparal (Tabs 5, 6; Newell 1947). This double-coverslip mount is subsequently inverted, so that the smaller coverslip is now at the bottom and placed on a drop of Canada balsam on a glass slide. This technique has been applied to mounts of algae, fungi,

mites, nematodes, trematodes, and sections (Diehl 1929; Newell 1947; Romeis 1948, p. 190; Mitchell & Cook 1952; Travis 1968; Kohlmeyer & Kohlmeyer 1972; Pritchard & Kruse 1982; Taft 1983; Jentzen 1984; Loveland & Centifanto 1986; Volkmann-Kohlmeyer & Kohlmeyer 1996). Nail varnish has been used as a barrier between glycerol and the xylene of Canada balsam or Caedax in order to prevent cloudiness in the periphery of the mount (Kohlmeyer & Kohlmeyer 1972; Volkmann-Kohlmeyer & Kohlmeyer 1996).

3.8.5 Corseal

Corseal consists of polystyrene, hard plastics, and solvents (Tabs 1, 8; Sabir 1996). Supposedly, it withstands a temperature range from -5°C to 60°C (Sabir 1996). Polystyrene dissolved in xylene after several steps of cleaning was suggested by Schmode (1989) as a mounting medium for microscope slides. Nothing is known about the long-term stability of such media, but the inclusion of polystyrene and hard plastics of unknown chemical composition puts a big question mark on the archival quality of these media. Horie (2011, p. 181) mentioned that polystyrene was sensitive to light and concluded that it "is generally too unstable [...] to be used on objects".

3.8.6 Glyptal

Glyptal is an enamel paint for insulating electrical applications and waterproofing car motor parts (Tabs 1, 8; Travis 1968) and comes in red, black, or transparent. This alkyd (originally alcid from alcohol and acid) is manufactured from a polyalcohol, viz, glycerol, and an acid, viz, phthalic anhydride, resulting in the polymer Glyptal (Jordão *et al.* 1996). Alkyd polymers polymerize slowly by oxidative cross-linking after evaporation of the organic solvents and become insoluble for solvents (Horie 2011, p. 265). The increasing number of cross-links seems to improve resistance to deterioration, because fewer unsaturated bounds remain available for oxidative degradation (Horie 2011, pp. 264–265). Alkyd resins are supposed to be quite resistant to hydrolysis but less so to UV light and extended exposure to salt solutions (Wypych 2013, pp. 357–358). Travis (1968) and Faraji & Bakker (2008) recommended Glyptal as a coverslip seal after 13 and five years of positive experience, respectively. Glyptal has also been suggested as adhesive and consolidant in paleontology (Brink 1957).

3.8.7 Glyceel (= Zut, Thorne ringing compound)

The ingredients of the later Glyceel for sealing glycerol mounts were mentioned already by Thorne (1935, p. 98; Tab. 1). Both Glyceel and Zut are not commercially available anymore, but Glyceel can be prepared in the laboratory using the recipe provided by Bates (1997; Tabs 8, 9). Zut withstood storage of glycerol mounts for at least 26 months at 66°C (Caveness 1969). Glyceel is reported not to "harden very well over a wax edge", but the senior author has never experienced this problem, probably because excess paraffin is scratched away and also removed with ethanol before applying the seal; also, the mount is ringed several times (Hooper 1986b, p. 317; Neuhaus pers. obs.). Glycerol mounts sealed with Glyceel in combination with a paraffin ring last at least 15 years (Tab. 14; Hooper 1986b; Neuhaus pers. obs.). Lactophenol mounts surrounded by a paraffin ring and Glyceel as coverslip seal last at least five years (Hooper 1986b). Probably, the predominance of carboncarbon bonds over carbon-oxygen bonds in the polymerized linseed oil used in the formula of Glyceel *versus* the raw linseed oil adds to the durability of the seal, because the carbon-carbon bonds are less prone to degradation than the carbon-oxygen bonds (Mills & White 1999, p. 41).

Both Glyceel and nail varnish contain cellulose nitrate, and this component has been identified in various adhesives as a weak component in terms of long-term stability (Koob 1982; Shashoua *et al.* 1992; Horie 2011, p. 214). Especially light accelerates breakdown of cellulose nitrate solutions (Ferreira & Combs 1951) as well as high temperature and relative humidity (Shashoua *et al.* 1992). In the face of these results, it appears quite amazing that Shashoua *et al.* (1992, p. 113) and Horie (2011, p. 216) stated that cellulose nitrate might "last up to 100 years". However, the explanation may lie in the fact that the polymer seems to be more stable if an appropriate plasticizer like diphenylamine is used, which also traps developing NO_x (Selwitz 1988, p. 42) and if the nitrogen oxides produced during oxidation and hydrolysis of cellulose nitrate can evaporate quickly and consequently not

accelerate the deterioration process (Horie 2011, p. 214). The latter is certainly the case if cellulose nitrate is used as an ingredient of a coverslip seal, where it forms a very thin layer directly exposed to the air. Shashoua *et al.* (1992) also identified "museum conditions in a temperate climate" as favorable for an expected lifetime of 50–100 years.

3.8.8 Liquid electrical tape

Liquid electrical tape was originally developed for insulating electrical connections on boats (Dornau *et al.* 1993). It has been suggested as coverslip seal lasting at least 20 years to the senior author in 1999 (John Holsinger in Könemann pers. com.) but found later to crack and to lose substance (Könemann pers. com.). Possibly, deterioration started because of loss of the plasticizer (Tab. 8).

3.8.9 Nail varnish

Nail varnish (= nail polish) was suggested as a satisfying coverslip by some authors (Tabs 8, 9; Hooper *et al.* 2005) but rejected by others, because the seal "shrinks and cracks excessively in time" (Esser 1973) or because "it lacks the body and plasticity of 'Glyceel'" (Hooper 1986a). The main reason for the shrinkage represents the diffusion of the plasticizer(s), especially camphor and dibutyl phthalate, out of the varnish (Figs 13, 14; Tab. 10; Horie 2011, p. 214). This should not take too long, because camphor sublimes at room temperature (Tab. 10; Shashoua 2008, pp. 41, 159; Horie 2011, p. 241). Expensive nail varnish may work much better than cheap varnish (Allington & Sherlock 2007a, 2007b). These authors also reported different sealing properties of two nail varnishes. Ronald Vonk (pers. com.) used transparent nail varnish for over two decades and suggested to keep slides "strictly in the dark". Still, some seals crack, whereas others do not, possibly because of the variable composition of the nail varnishes over time (Vonk pers. com.). In any case, it can be expected and has been reported (Cushing 2011) that a manufacturer's formula for a nail varnish will change over time and thus, inadvertently, influence the varnish's sealing properties. For further discussion see chapter 3.8.7 Glyceel (= Zut, Thorne ringing compound).

3.8.10 Norland Optical Adhesive 61 (= NOA 61)

The Norland Optical Adhesive 61 (= NOA 61) represents a polyurethane and consists of a proprietary mercapto-ester and triallyl isocyanurate (Tab. 8). Triallyl isocyanurate is liquid and polymerizes after UV radiation; it has a high potential for cross-linking (http://www.micchem.com/triallyl_isocyanurate.html, accessed 27 January 2016). NOA 61 seems to adhere well to glass surfaces, offers low shrinking of 1.5% on curing, does not remain tacky after curing in areas exposed to air, and has a shelf-life of four months if stored at 5–22°C (http://www.norlandprod.com/adhesives/NOA%2061.html, accessed 27 January 2016; Taylor 2005). A detailed description of how to make a total mount of a rotifer in glycerol on a glass slide and how to seal the mount with NOA 61, the silicone rubber Dow Corning® 3140 RTV Coating, and finally asphaltum varnish is given by Jersabek *et al.* (2010). Originally, Clearseal One and Bioseal Mountant Two (Northern Biology Supplies, UK) were used instead of the silicone and asphaltum (Taylor 2005). The company does not seem to exist anymore (Neuhaus pers. obs.). Jersabek *et al.* (2010) reported no changes in microscope slides in eight years.

3.8.11 Paraffin

Candle wax (Romeis 1948, p. 192; Goodey 1957), beeswax plus paraffin wax (Schmölzer 1960), paraffin wax plus petroleum jelly (Doncaster 1962; Hooper 1986a, pp. 76–77, 79), paraffin wax (Hooper 1986a), and soft paraffin (= petroleum jelly, vaseline) (Spence 1940b) were used alone or in combination with other seals to stabilize a glycerol mount and to prevent evaporation of glycerol or other liquid mounting media. Paraffin was introduced about 1869 as an embedding medium for histological sectioning of biological specimens (Sanderson *et al.* 1988). The medium consists nowadays of a mixture of saturated hydrocarbons with a chain length of 20–35 carbon atoms and a variety

of more or less proprietary additives like beeswax, crepe rubber, dimethyl sulfoxide, and synthetic polymers like polyisobutylene (Böck 1989, pp. 123–124; Sanderson 1994, p. 113; Kiernan 1999, p. 44, 2015, p. 53). Paraffin melts at 52–58°C and should not be heated too much above this temperature in order to avoid potential degradation of additives and yellowing of medium (Böck 1989; Sanderson 1994; Kiernan 1999, p. 44, 2015, p. 53).

Soft paraffin was poured with a hot spoon on a glass slide to an almost finished mount. This soft paraffin was supposed to be inert to the water of liquid aqueous preparations (Spence 1940b). However, the soft paraffin melted in unfavorable climates like in the USA, but mounts also leaked in the British climate (Spence 1941b). In a more historical way of transferring paraffin wax in a circle to a glass slide, a bent wire wrapped with a cotton thread was used (Green 1926); alternatively, the wick of a paraffin-petroleum jelly mixture was "lit, allowed to saturate with molten wax and then extinguished and used as a brush for applying the cover glass seal" (Doncaster 1962; see also Romeis 1948, p. 192). Schmölzer (1960) suggested melting beeswax and paraffin wax in a glass tube and pressing it out in a circle on a coverslip. Maeseneer & d'Herde (1963) improved the way of creating a paraffin wax ring on a slide by using a tube dipped into molten paraffin instead of a candle or an analogous device. Nowadays, a glycerol-paraffin mount is prepared by dipping a metal cylinder into a dish with molten paraffin at about 60°C on a hotplate and immediately on a glass slide or on a rectangular coverslip (Fig. 27B; Tab. 13: Technique E; Hooper 1970, p. 50, 1986a, pp. 76–77; Hooper *et al.* 2005, pp. 70–71). The subsequent mounting process including ringing with Glyceel is summarized in Table 13 (Technique E). If the temperature of the hot plate is too low, the paraffin will not melt entirely in the Petri dish and develop a skin. Too much heat will result in oxidation and consequently yellowing of the paraffin and loss of "much of its crystaline structure" (Sanderson 1994, pp. 46–47).

3.8.12 *p*-Phenyl phenol formaldehyde resin

p-Phenyl phenol formaldehyde resin sold as the commercial varnish TufOn™ 74 until about 1963 (Cushing 2011) was suggested for sealing liquid mounts containing tung oil and a metallic soap as oxidizing agent dissolved in xylene (Barghoorn 1947). The polymerized but elastic seal is resistant to a variety of organic solvents and mineral and organic acids. It withstands accelerated aging at 50°C for 8 months with repeated changes to room temperature. However, the unused sealant should be stored in quantities of at least 250 ml, because it rapidly starts polymerizing in small quantities exposed to air (Barghoorn 1947). Possibly for this reason, the resin does not seem to have been widely accepted.

3.8.13 Sealing wax varnish

Sealing wax varnish contained mainly natural resins like gum colophony, Venetian turpentine, and shellac (Figs 10, 11, 21, 26; Tab. 8; see also chapter 3.8.14 Shellac). Colophony oxidizes and darkens within a short time, becomes brittle because of its small molecular size, and sensitivity to water increases (Heesters *et al.* 2002; Scalarone *et al.* 2002, p. 348; Horie 2011, p. 248). Venetian turpentine also becomes brittle when dry (Mills & White 1999, p. 102). Probably for these observations, older recipes included beeswax (Gray 1954, p. 651), which may act as a plasticizer. Picture varnishes made of natural resins become brittle over time and crack (Scalarone *et al.* 2002), which indicates a limited life-time for coverslip seals made of natural resins.

3.8.14 Shellac

Shellac is secreted by the hemipteran *Kerria lacca* (Kerr, 1782) and other related species parasitizing different tree species in India, Thailand, and southeast Asia (Colombini *et al.* 2003; Buch *et al.* 2009, p. 694). The stick lac secreted by the animal on the twigs of the host plant is cleaned by crushing, sieving, and washing in water to give seed lac, and the latter is further processed to button lac or shellac either by (1) melting resulting in orange shellac of variable quality used mainly for technical applications, or by (2) bleaching with sodium hypochlorite and de-waxing resulting in bleached shellac or white shellac of variable quality used mainly in food industry and for pharmaceutical purposes, or by (3) the more gentle solvent extraction with ethanol, filtering, and often treatment with activated carbon resulting in de-waxed shellac used also in microscopy (Fig. 26; Gray 1954, p. 653; Frison

1955, p. 204; Mills & White 1999, p. 115; Colombini *et al.* 2003; Buch *et al.* 2009; Sutherland & Río 2014). Chlorine is chemically incorporated into bleached shellac "which does not, *a priori*, seem a sound idea as regards durability" (Mills & White 1999, p. 117). Analysis of physical and chemical parameters reveal that the properties of shellac depend on the insect species, the host plant species, parameters of processing, storage conditions, and age, so different shellac grades and previous treatments can be distinguished (Colombini *et al.* 2003; Buch *et al.* 2009, pp. 694, 701; Giri *et al*. 2010; Sutherland & Río 2014).

Shellac belongs to the "resin" fraction of sticklac, which consists of 70–80% "resin", 48% coloring chemicals, and 6–7% "wax" (Mills & White 1999, pp. 116–117). The resinous part is mainly composed of aliphatic and alicyclic (sesquiterpene) acids as well as their oligomers and polymers, which are polyesters; the "soft resin" components are ether-soluble, whereas the "hard resin" fraction is not (Fig. 26; Tab. 8**;** Colombini *et al.* 2003; Buch *et al.* 2009; Sutherland & Río 2014). The alicyclic jalaric acid and laccijalaric acid may undergo autoxidation because of their aldehyde and alkene groups, resulting in elevated levels of laccishelolic acid, shellolic acid, and their epimers; however, laccishelolic acid, laksholic acid, shellolic acid, their epimers, and traces of chlorinated substances may result from alkali treatment during previous commercial processing (Fig. 26; Mills & White 1999, p. 117; Colombini *et al.* 2003, pp. 360, 362; Sutherland & Río 2014, pp. 155, 158). Bleached shellac "is known to develop problems such as insolubility with age [...] likely related to the presence of residual chlorine" (Sutherland & Río 2014, p. 157). Opposite to aleuritic acid and the alicyclic components of shellac, butolic acid is supposed to represent one of the few molecules not impacted by ageing, so it can be used to identify shellac chemically in older samples (Fig. 26; Colombini *et al.* 2003, p. 363), but Sutherland & Río (2014, p. 158) stated that butolic acid "may not always be present at detectable levels, and related compounds such as 8-hydroxy myristic and 8-hydroxy palmitic acids should be searched for as well".

Shellac seems to have been applied in various recipes and under different names such as brown cement, which seems to be a solution of shellac in ethanol (Tab. 8; Garner & Horie 1984, p. 98). Shellac dissolved in petroleum ether to which rubber or gutta-percha is added makes a coverslip seal named marine glue (old formula from 1880) and is supposed to be extremely water-resistant (Tab. 8; Gray 1954, pp. 22, 655). Alternatively, a mixture of shellac and gutta-percha in Venetian turpentine, also named gutta-percha cement, may represent the original recipe of marine glue (Gray 1954, pp. 651, 656). Additional recipes of shellac cements are given in Gray (1954, pp. 652–656), but are less recommended by that author. De-waxed shellac has been used explicitly for microscope slides at least in some recipes (Gray 1954, p. 653; Frison 1955, p. 204), because the wax would reduce adhesion (Horie 2011, p. 259). Shellac "is not completely water resistant" (Feller 1971, p. 121). A shellac coverslip seal cannot be dissolved again readily in alcohol at room temperature for re-mounting, but heating may result in dissolution (Mills & White 1999, p. 117; Horie 2011, pp. 258–259). This may be due to increased cross-linking over time (Colombini *et al.* 2003; Coelho *et al.* 2012).

3.8.15 Silicone rubber

Silicone rubber was diluted with petroleum ether in order to receive a sufficiently fluid rubber for application as a seal (Fig. 12; Tab. 8); the cured seal withstood storage of glycerol mounts for 26 months at room temperature and also at 66°C (Caveness 1969). Jersabek *et al.* (2010) proposed to apply the silicone rubber Dow Corning® 3140 RTV Coating outside a ring of NOA 61 and finally cover the mount with asphaltum varnish. Generally, silicone rubber is supposed to last for at least 20 years (Petrie 2007, p. 443). It has not been investigated whether or not the acetic acid released during curing (= acetoxy cure system) of the rubber (Petrie 2007, pp. 443, 445) influences the stability of the mounting medium. Silicone rubbers with an acetoxy cure system accumulate dirt particles over time (Petrie 2007, pp. 443, 445). Also, silicone rubbers seem to possess a "high oxygen and water transmission rate" (Horie 2011, p. 276; also Petrie 2007, p. 446). Several percentages of water absorption are common for silicone polymers (Wypych 2013, p. 505). Thus, silicone rubbers may stabilize a mount mechanically, but may not prevent gases entering the mounting medium as well as water entering or leaving the mount. Silicones are also vulnerable to UV degradation (Wypych 2013, p. 503). A word of warning must be uttered here, because silicone rubber is extremely difficult to remove totally from any surface and requires a special cleaning fluid for removal (Horie 1983, p. 3-3), which may or may not work sufficiently (Horie 2011, p. 280). In addition, un-reacted silicone oil from the rubber will migrate over time (Davison 2003, p. 218; Horie 2011, p. 281) from the seal and may affect the mounting medium and even interact with the specimen.

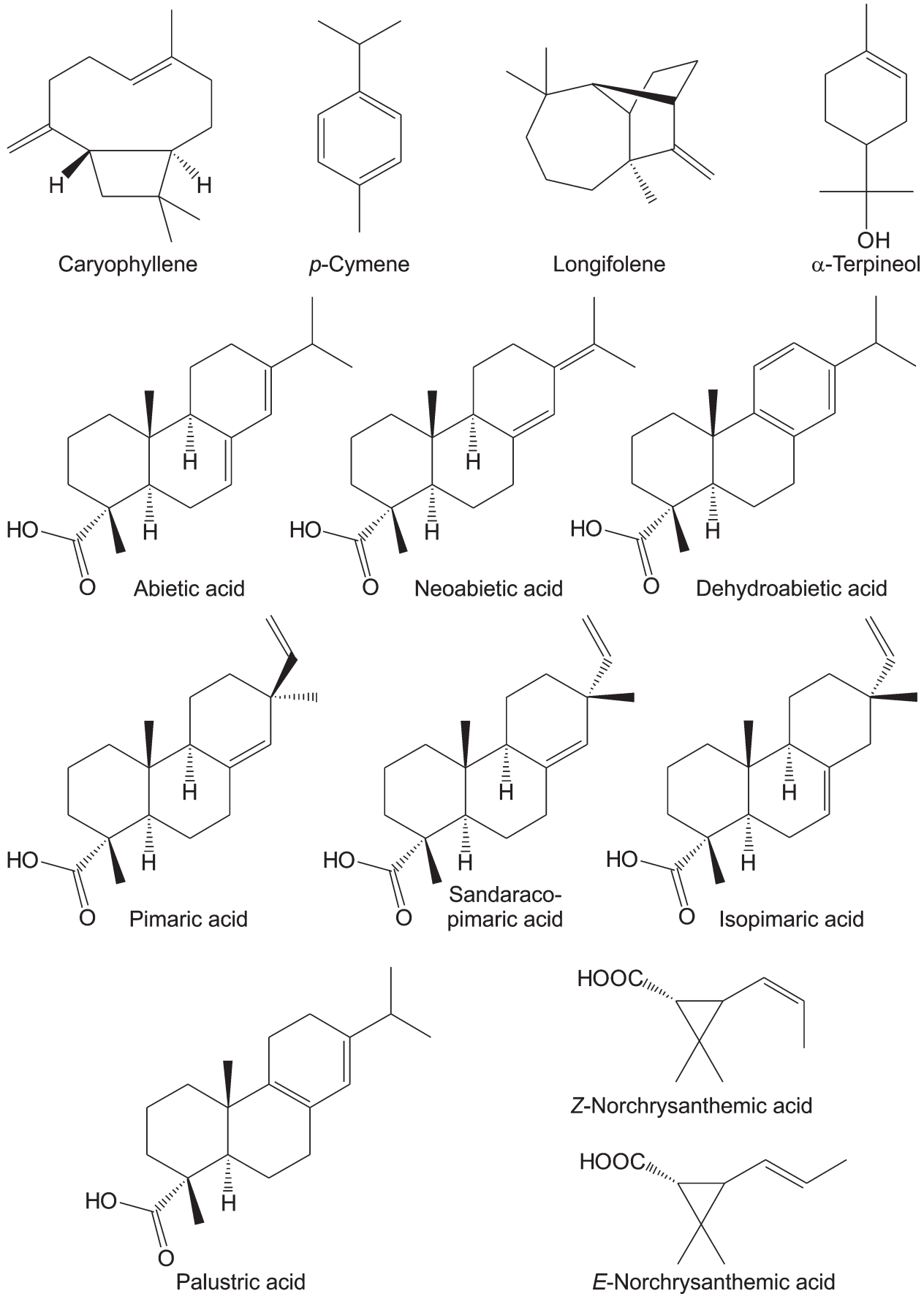


FIGURE 21. Chemical structure of the ingredients of gum colophony (= rosin, Greek pitch) from various species of *Pinus*.

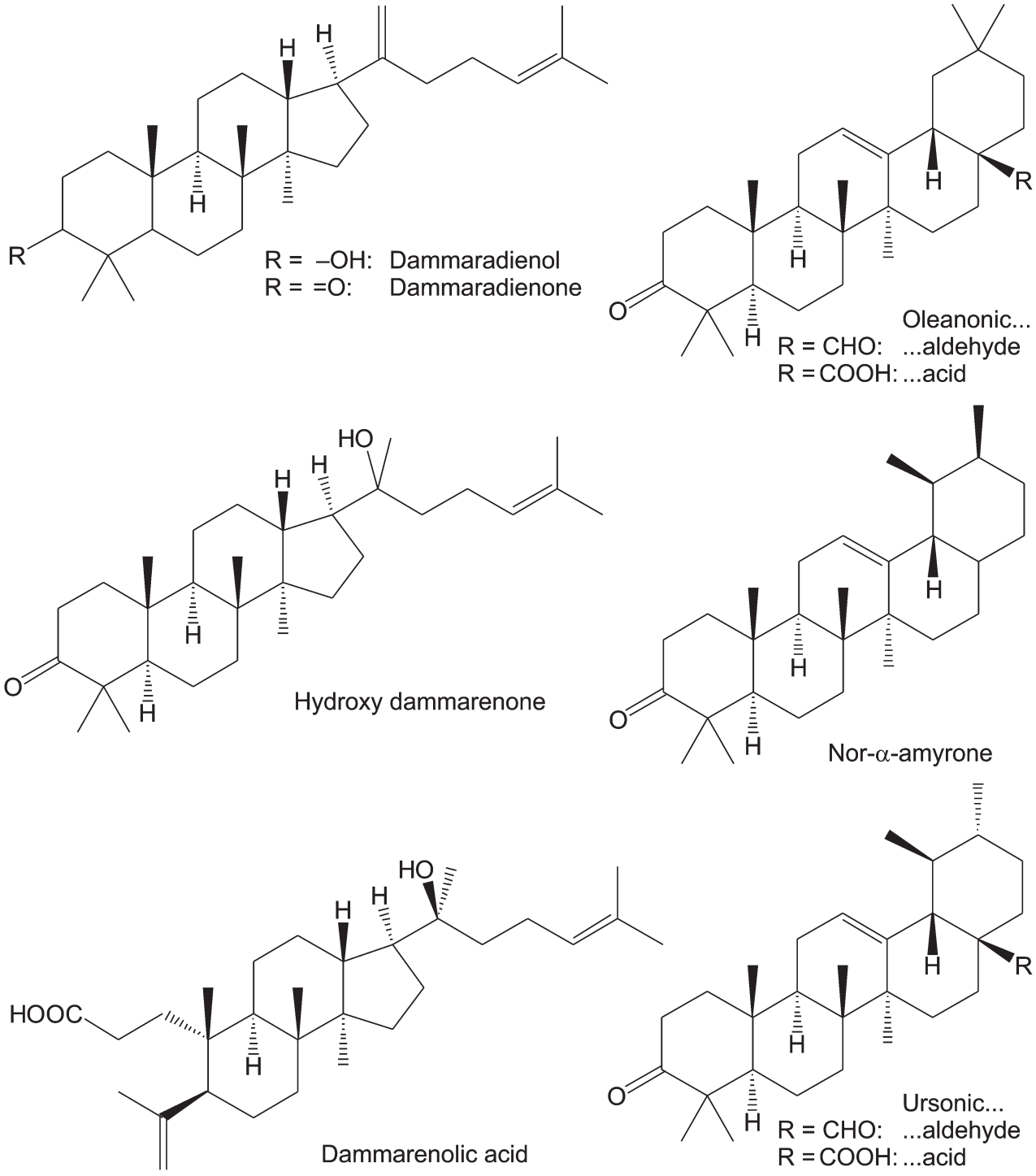


FIGURE 22. Chemical structure of the ingredients of gum dammar from an unknown species.

3.8.16 White lacquer

In the 19th century, a mixture of gum dammar and zinc oxide (Fig. 22; Tab. 8) was frequently used as a coverslip seal finished by a 'gold' paint containing brass pigments (Behrens 1892, p. 73; Garner & Horie 1984, p. 93). The finishing varnish seems to be necessary in order to protect the seal against dirt (James 1887, p. 95). The interaction of shellac and zinc oxide in varnishes leads to "the formation of metal soaps and other by-products such as oxalates" (Poli *et al.* 2014, p. 143). These results may also indicate a negative effect on the longevity of a coverslip

seal for the combination of zinc oxide with the natural resin gum dammar. Behrens (1892, p. 73) mentioned as one ingredient also gum mastic, so probably different recipes existed for a medium named white lacquer.

Dammar resins are produced by about 500 tropical species of the Dipterocarpaceae, but the resin gum dammar used in Europe and North America seems to originate only from several species of *Hopea* or *Shorea* (Mills & White 1999, p. 107). Gum dammar yellows and cross-links increasingly with age and requires increasingly more polar solvents for at least partial chemical removal; mechanical removal of the gum has to be considered if parts of the resin cannot be dissolved by organic solvents anymore because of the cross-linking. Also, the resin becomes more brittle with age (Horie 2011, pp. 253–257). UV light leads to rapid oxidation of gum dammar, but a hindered amine light stabilizer may extend "the useful lifetime (assessed by yellowing and solubility) potentially up to 136 years" for picture varnishes (Horie 2011, pp. 257).

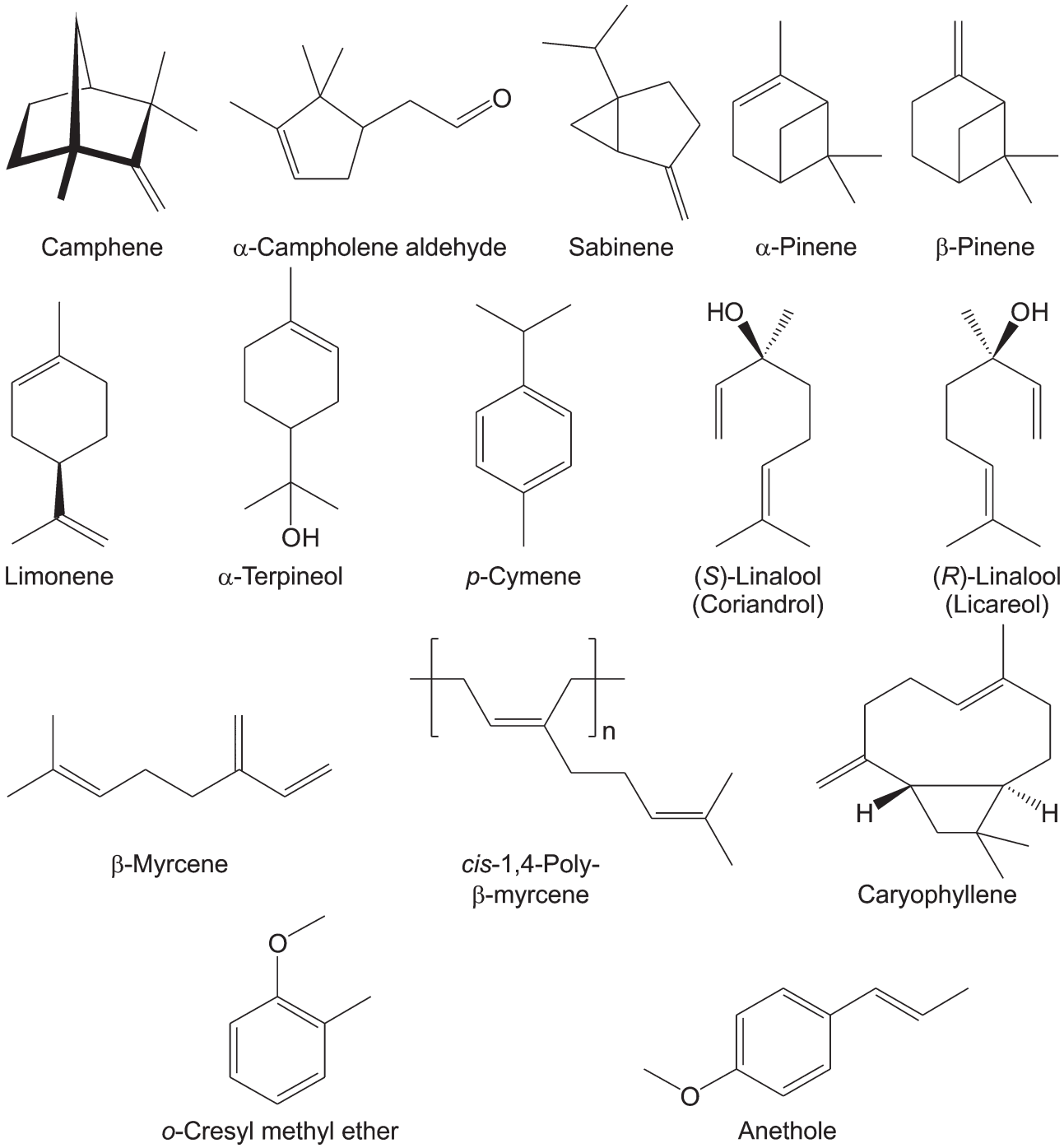


FIGURE 23. Chemical structure of monoterpenes, seaquiterpenes, and more volatile ingredients of gum mastic from *Pistacia lentiscus*.

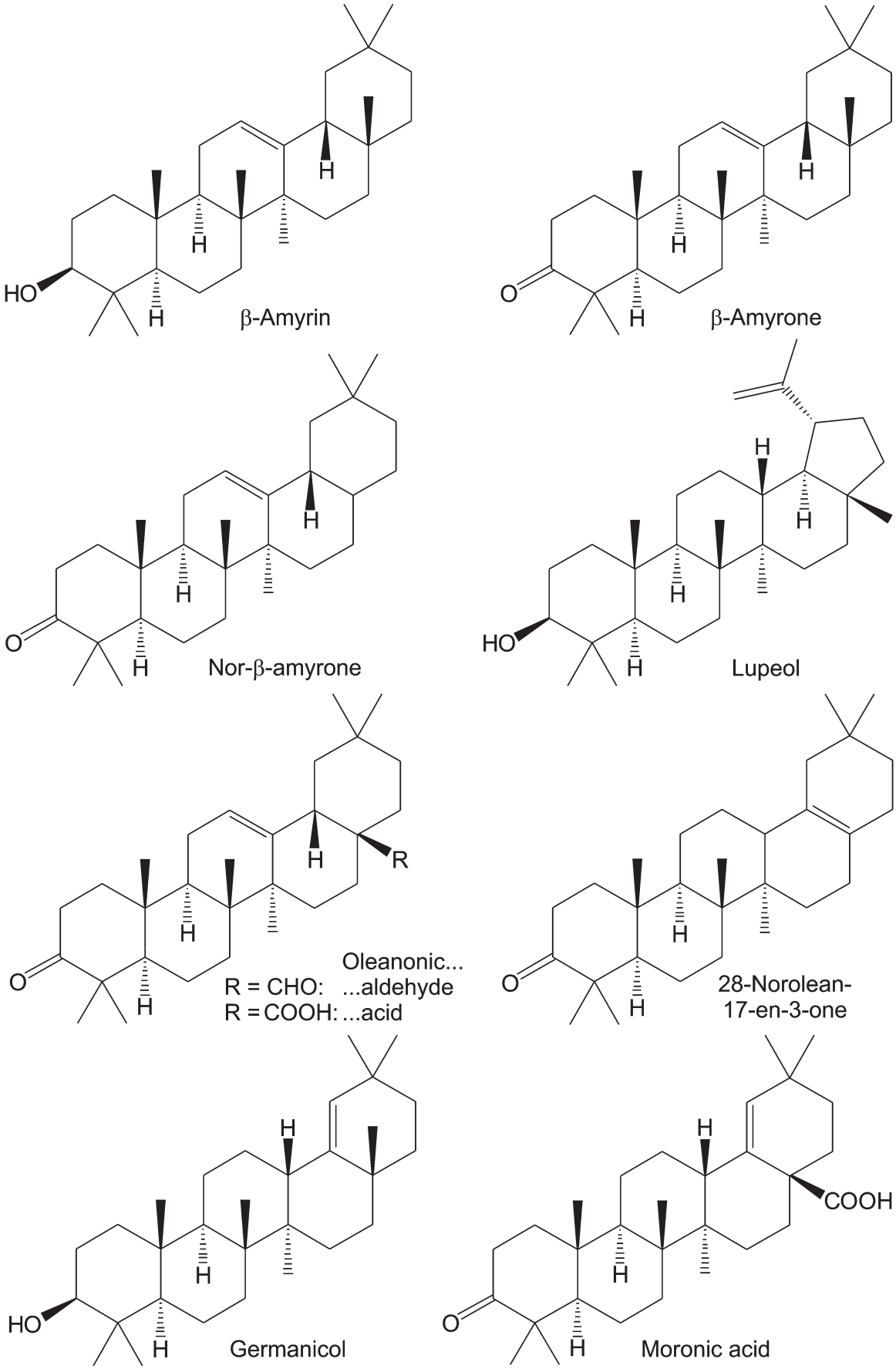


FIGURE 24. Chemical structure of triterpenes of gum mastic from *Pistacia lentiscus*, part 1.

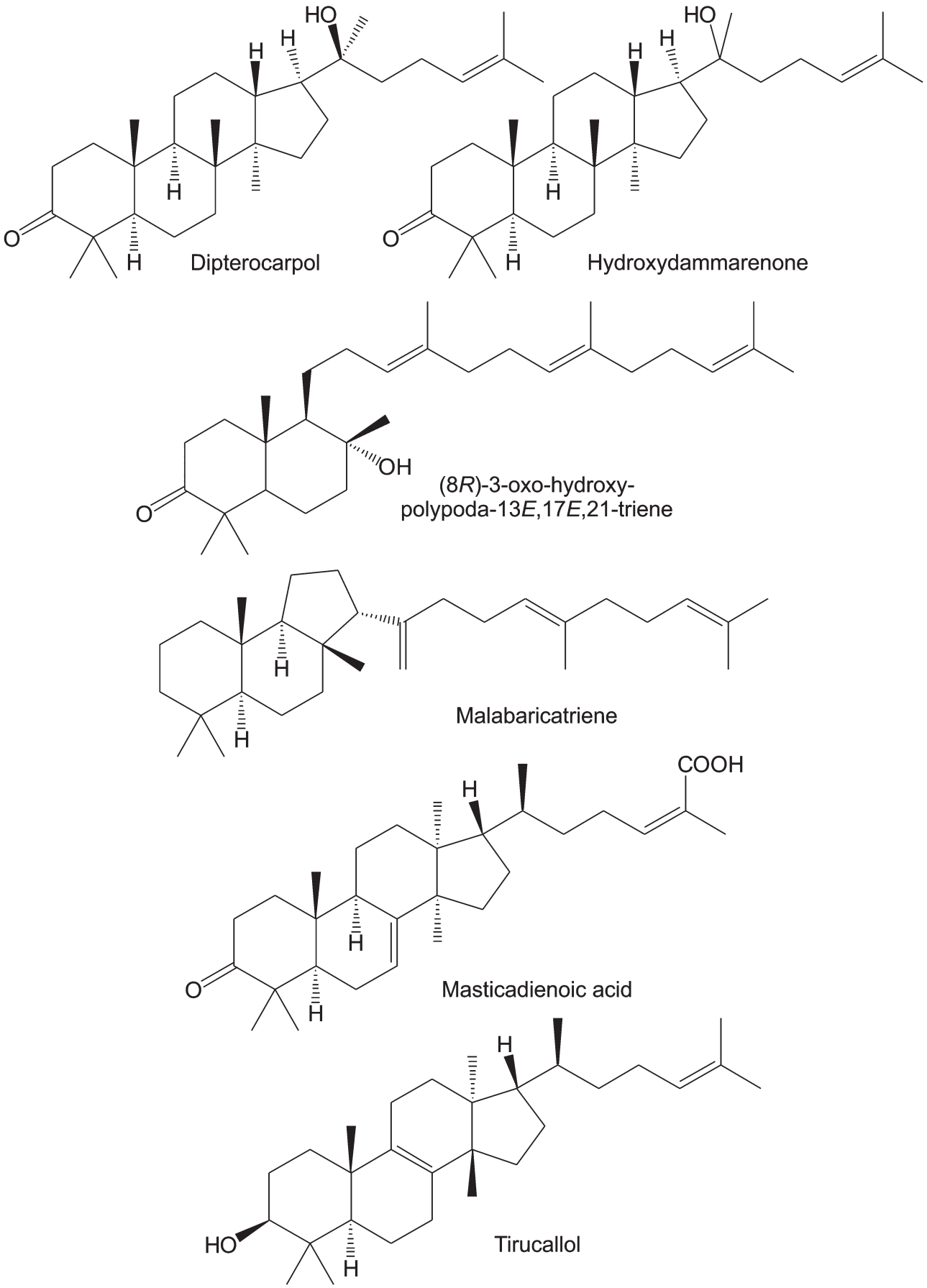
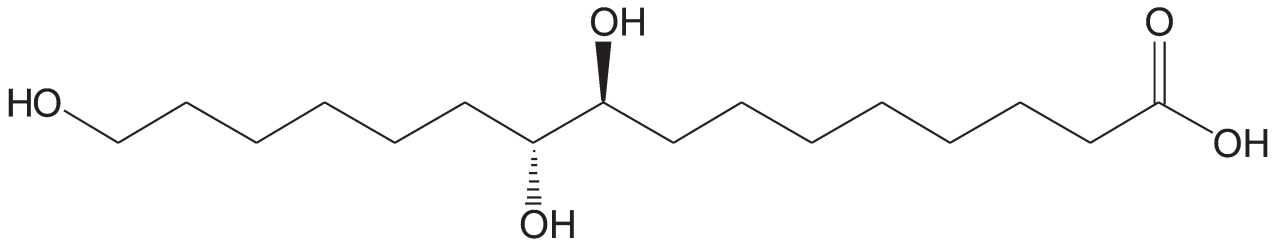
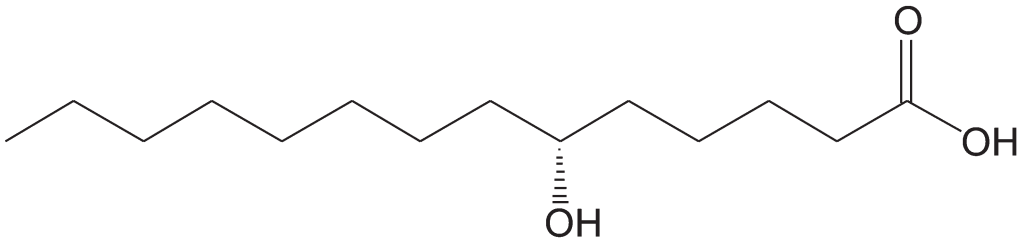


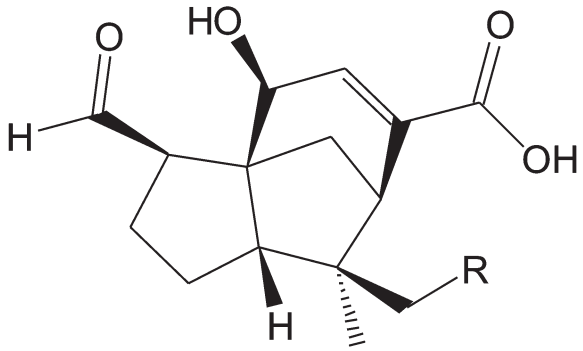
FIGURE 25. Chemical structure of triterpenes of gum mastic from *Pistacia lentiscus*, part 2.



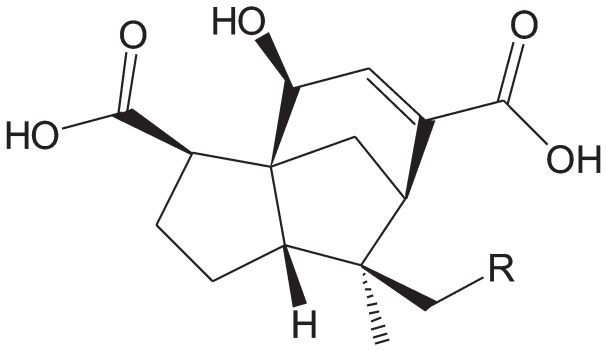
Aleuritic acid



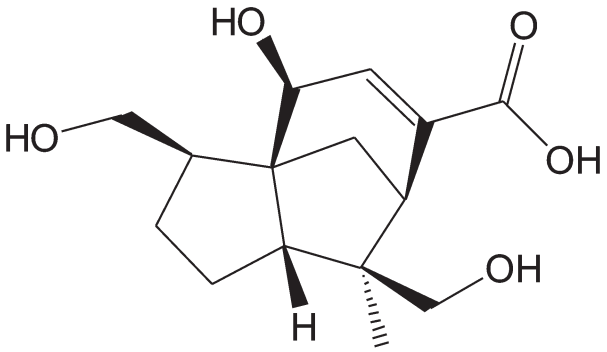
Butolic acid



R = OH: Jalaric acid
R = H: Laccijalaric acid



R = OH: Shelloic acid
R = H: Laccishelloic acid



Laksholic acid

FIGURE 26. Chemical structure of ingredients of shellac from the hemipteran *Kerria lacca* and related species.

3.9 Labels

3.9.1 General aspects of labels

Identification and location data is documented on microscope slides on labels of all kinds of paper qualities with all kinds of inscriptors like pencil, ball-point pens, felt-tip pens, and ink (Figs 2A–H, 3A–K, 4A–K, 5A–J, 15A, B, E, F) as well as with a "permanent" marker (Fig. 5H), ink inscription covered with a thin layer of mounting medium (Fig. 5G) or not, and inscription with a diamond-tipped or tungsten carbide-tipped engraving scribe (Figs 2A, F, 3A–D; Adam & Czihak 1964; Hooper 1986b; Gütebier 2011). It is strongly recommended to write data as completely as possible in natural language without abbreviations or codes, the decipherment of which may not be available to others or even may get lost (Levi 1966, p. 187; Martin 1978, p. 110; Notton 1995; Amrine & Manson 1996, p. 391; Huber 1998, pp. 368, 375). The month of any date should be either abbreviated or given in roman numerals in order to avoid confusion between the American and Canadian/European way of writing dates (Levi 1966, p. 187; Martin 1978, p. 111). The left label should bear information about the determination, life history stage, and as specific as possible information about the specimen preparation like fixation, chemical maceration, mounting medium, and coverslip seal, whereas the right label should harbor the location and collecting data (including geographic location, latitude, longitude, altitude or depth, host and host organ if applicable, collector, collecting gear if applicable, and date of collection) ¾or *vice versa* (Mound & Pitkin 1972; Krantz 1978, p. 90; Martin 1978, pp. 109–112; Palma 1978; Pritchard & Kruse 1982; Amrine & Manson 1996; Brown 1997, p. 5; Walter & Krantz 2009, fig. 7.2). A slightly different labelling system was proposed by Robinson (1976). Machine-readable barcodes may be added but will inevitably suffer from a limited life-time of the bar code reading hardware and the related software version and depend on the longevity of the adhesive, with which the barcode label is glued to the microscope slide. So a barcode may complement a label in natural language but cannot really replace it. Information about the specimen preparation including the recipe of a home-made mounting medium may not fit on the labels on the slide, so this should also be documented in the registry catalogue or database but rarely is. Such data may be extremely valuable in case a slide has to be restored.

3.9.2 Label paper

It is strongly recommended to use archive-quality paper or medium-thickness cardboard according to DIN EN ISO 9706 (Tab. 14), because such paper consists of unbleached cellulose and does not contain recycled fibers, lignified fibers, acids, and optical brighteners (Hawks & Williams 2005; Kilby 1995; Sturm 2006). At the same time, such paper is buffered with calcium carbonate to a pH of 7.5–10. Alternatively, paper should be used that has passed the PAT-test (= photo activity test), which is used for archiving negatives. It differs from the former paper in that it has a neutral pH but is unbuffered. One reliable source of label paper in Germany for slides and cardboard for Cobb aluminum slides is Klug Conservation, which also provides certificates for its products (Tab. 12). The Museum für Naturkunde Berlin uses since 2016 their buffered "Museums- und Fotoarchivpapier mit Alkalipuffer, altweiß" with a weight of 120 or 160 g/m² fulfilling standards DIN EN ISO 9706 (information and documentation about resistance to aging of paper-based documents), ANSI / NISO Z.39.48 (American National Standard for Permanence of Paper for Publications and Documents in Libraries and Archives), DIN 6738:2007 (highest life expectancy class LDK 24-85), and DIN ISO 16245 (information and documentation about covers and boxes used for long-time storage of paper-based documents).

Brown & Boise (2005, 2006) used cardboard for labels or glue label paper to a piece of cardboard and this to a glass slide with pH-neutral Lineco PVA adhesive. Removal of such labels is done by soaking in water or by mechanically cutting the cardboard horizontally with a scalpel. The advantage of lateral cardboard labels on a standard glass slide results in some protection of the centrally mounted coverslip against mechanical damage if slides are stored vertically or stacked horizontally (Wagstaffe & Fidler 1955, p. 198–199; Eastop & Emden 1972; Brown 1997; Brown & Boise 2005, 2006). This kind of storage is not recommended here for the reasons given in chapter 3.2 Storage. Also, high power magnifying objectives of the microscope with a low working distance may bump into thick pieces of cardboard. Therefore, paper or medium-thickness cardboard labels are preferred over thick cardboard labels.

TABLE 12. Lab equipment at one workplace for mounting and restoring microscope slides.

Lab equipment	Purpose	Source
exhaust tube connected with hood	minimizing harm to staff	
hotplate	soaking slides in solvent; melting paraffin	
oven	drying mounted slides	
stereo microscope on swingarm stand and heavy base	observation of slides on hotplate	
cold-light source and 2 flexible fiber optic light guides	proper illumination of slide on hotplate	
compound microscope equipped with DIC	immediate control of mounted specimen	
blotting cardboard, 450 g/m², # 2402	wet poultice for soaking label on slide before mechanical removal; drying labels	http://www.gsk-conservation.de/en_materialien.htm
Hollytex polyester fleece, 80 g/m², # 37 265	transfer of labels, non-reactive with glue	http://www.gmw-shop.de
absorbent purified cotton wool DAB 6, e.g., Augenwatte from Kerma	cleaning of glass surfaces; removal of glue from label	http://www.kerma.de
Whatman lens cleaning tissue 105	cleaning of glass surfaces	
polyester swabs ITW Texwipe CleanTips®	cleaning of glass surfaces	http://www.texwipe.com
museum paper white 120g/m², A4, # 017120, fulfills norms DIN EN ISO 9706: 2010, ANSI/NISO Z.39.48: 1992, DIN 6738: 2007	new labels; small label with catalogue number accompanies slide in Petri dish	http://www.klug-conservation.com
standard glass slide, hydrolytic class 3 soda-lime glass, cleaned, extra white, free of surface defects, inclusions, streaks, and bubbles, with ground edges	for conventional microscope slides	http://www.menzel.de
coverslips No. 1 (0.13–0.16 mm), hydrolytic class 1 borosilicate glass D 263™, extra white, highly transparent, colorless, free of blisters and flaws	circular (15 mm diameter) for glass slides; circular and 24 mm x 24 mm for double-coverslip mounts	http://www.menzel.de
Cobb aluminum slide	frame of double-coverslip mounts	local metalworking firm
turntable, brush	ringing coverslips with coverslip seal	
cardboard trays for slides	short-time storage of slides	
scalpel, needle mounted on stick	removal of coverslip seal and top coverslip	
blow ball, fine brush, disposable soft lab tissues	cleaning of slides	
pipettes	adjusting amount of solvent in Petri dish	
forceps	manipulation of coverslips & larger specimens	

……*continued on the next page*

TABLE 12. (Continued)

Lab equipment	Purpose	Source
skewers	manipulation of coverslips; cleaning	
Irwin-loop, fine needles mounted on holder	transfer of small specimen to and from slide	
Petri dishes, watch glasses	soaking slides in solvent and keeping label with slide	
diamond- /tungsten carbide-tipped engraving scribe	engraving catalogue number on slide	
pencil	writing on label	
light- and ethanol-proof pigment ink (see Tab. 16)	permanent writing on label	
2 coated wooden plates, weight	drying labels	
Klucel G®	adhesion of label to slide	http://www.gmw-shop.de
Araldite 2020	epoxy resin for adhering broken glass slide	
Bookkeeper™ in pump spray application; water-based solution of calcium hydroxide in tray	deacidification of acidic labels	http://www.ptlp.com/en
glycerol in dropping bottle (stored in desiccator), paraffin	for glycerol-paraffin mounts	
small Petri dish with paraffin, metal tube	for making paraffin ring on slide or coverslip	
Canada balsam, Euparal, ...	mounting medium	
Glyceel	coverslip seal	Bates 1997
distilled water, ethanol, xylene	cleaning; solvents for mounting medium	

3.9.3 Inscribing

In any case, the catalogue number or any other unique identifier in natural language should always be scratched in with a diamond-tipped engraving scribe, a tungsten carbide-tipped engraving scribe (e.g., Bel-Art, Glascribe F44150), or an electrical engraving tool somewhere on every slide, so this most important cross-reference can never be separated from the slide specimen (Figs 2A, F, 3A–D; Tab. 16; Essig 1948; Pritchard & Kruse 1982; Sanderson 1994, p. 55; Brown 1997, p. 5). Inscription with a diamond-tipped or tungsten carbide-tipped engraving scribe undoubtedly represent the most durable documentation while at the same time not easy to read at a glance. Certainly, the least recommendable documentation is to write directly on the glass slide using a pencil on the frosted area of the slide or a "permanent" marker resistant to water and organic solvents and lightfast but vulnerable to mechanical abrasion (Fig. 5H). In most cases, a paper label will do best in terms of permanence and readability of the label. Certainly, ball-point pens and felt-tip pens do not belong to the permanent inscriptions, because they may be easily dissolved or blurred by immersion oil (Hooper 1986b) or solvents (Neuhaus pers. obs.).

It is most convenient to write labels with a laser printer (Tab. 14). In the printing process the toner of a laser printer is heated and pressed on the paper surface. In case the toner is shown not to be resistant enough to mechanical abrasion, e.g., by rubbing the letters on the paper with a finger, the label may be heat-treated in an oven or with an iron (heat level 1) before glueing it on the slide; if too much heat is applied, the toner will remain with the iron instead of the paper (Neuhaus *et al.* 2012). Good results over two decades with disposable ink pens for handwriting are available for Edding profipen 1800 (Tab. 14; Neuhaus *et al.* 2012). Other pens like Faber-Castell ecco pigment also show good results over several years (Neuhaus pers. obs.). Sturm (2006, p. 50) suggested as ink for hand writing Rotring 17 black, Hunt speedball super black ink, Pelikan 17 black, Pelikan 50 special black, and Higgins T-100 (Tab. 14). Heikinheimo (1988, p. 40) recommended to let any ink dry for at least 5 minutes before further handling the label; this is strongly encouraged by the senior author. Ink jet printers are generally not recommended for generating labels, because most "inks tend to fade on exposure to light" and are "soluble in water or organic solvents"(Sturm 2006).

3.9.4 Glue

Paraloid™ B-72 (USA: Acryloid B-72) seems to have been supplied in various formulae such as poly(methyl methacrylate-co-ethyl acrylate), poly(ethyl methacrylate-co-methyl acrylate), and poly(ethyl methacrylate-co-methyl acrylate-co-butyl methacrylate) (Feller 1971, p. 125; Horie 2011, p. 159). The polymer dissolved in organic solvents such as ethanol, acetone, toluene, and xylene fulfills the highest standards in long-time stability with an expected life-time of over 100 years and removability by organic solvents (Horie 2011, p. 159; Davidson & Brown 2012). It is commonly used in art conservation as picture varnish and glass adhesive and nowadays also in vertebrate paleontology, Davidson & Brown (2012) provide an overview of the properties of this polymer. Paraloid™ B-72 was suggested by Gütebier (2011, p. 74) for attaching labels to glass slides. Before application to a label, it must however be checked whether or not the inscription on the label may be dissolved by the solvent of the adhesive.

In order to avoid exposition to harmful organic solvents we suggest to adhere labels to the slide with an archive-quality, water-removable adhesive containing either wheat starch or hydroxypropyl cellulose like in Klucel G® as recommended by the paper conservator Dirk Schönbohm at the Museum für Naturkunde Berlin (Neuhaus *et al.* 2012) and also used successfully among other adhesives at the British Museum (Shashoua & Rugheimer 1998) and elsewhere in conservation (Butler & Klug 1980; Horie 2011, p. 211). The adhesive is sold in Japan under the name Nisso HPC® by Nippon Soda Co. Ltd. (Butler & Klug 1980). Klucel G® seems to possess excellent photochemical stability but loses its degree of polymerization over time, which may be beneficial for the reversibility of the adhesive; therefore, Gill & Boersma (1997) suggested a stability of 20–100 years for this polymer in textile restoration. Klucel G® is soluble both in water and ethanol below 38°C; a 7% solution in water with 5% ethanol is recommended for mounting labels on slides. Adhesives containing wheat starch or hydroxypropyl cellulose work well with both paper and glass surfaces. Methyl cellulose paste can be used to connect a label with a piece of cardboard (Horie 2011, p. 211) of a Cobb aluminum slide, but does not adhere well to a glass surface (Hawks & Williams 2005). The major drawback of water-soluble adhesives is the vulnerability of the slide-label system to a

disaster in a museum collection involving water, such as breakage of a pipe and fire (Baker & Morse 2001). Least advisable are gummed labels or self-adhesive labels (Fig. 5I, J), because these very rarely consist of archive-quality components (Essig 1948; Brown 1997). Also, the glue may migrate through the paper to the surface of the label (Fig. 2G).

3.9.5 Storage of old labels

Old paper labels removed from a slide should never be discarded as suggested by Wilson (1971), because they contain usually some handwritten information, which may assist in identifying the person, who wrote the notes. Also, the design of the label may allow one to draw conclusions about which time a label (and the corresponding slide) may have been made. If a label has to be removed from a slide, it should either be glued back on a slide or be stored in a separate place. Before final storage, a label should be deacidified (see chapter 3.10.3 Restoration of slides—labels; Fig. 27F). Advised by a paper restorer (Neuhaus *et al.* 2012), collections of the Museum für Naturkunde Berlin store labels, sorted by taxonomic group and catalogue number, either in 4-flap paper envelopes filed in cardboard boxes (Fig. 27E) or alternatively in polyester slide and negative film preserver sheets produced by Secol housed by a cardboard file system made by Klug Conservation (Fig. 27F, G). All materials are of archive-quality, and the polyester preserver sheets have passed the PAT-test (see chapter 3.9.2 Label paper). The 4-flap paper envelopes offers the advantage that new labels can be included in the box system without having to move neighboring labels, but suffer from the more time-consuming procedure of labelling each envelope on the outside and folding the four flaps both for storage and for subsequent reading (Fig. 27E). The slide and film preserver sheets allow the user to see every label at any time at a glance, but may require the curator to move labels if a new label requires a position between already stored labels. The latter may be overcome by deliberately leaving positions empty. Also, a microclimate may develop in the polyester pockets of a sheet. A piece of paper in each pocket behind a label may allow some environmental exchange (Fig. 27G).

3.10 Restoration procedures

3.10.1 General considerations for restoration

In view of the limited resources of staff in probably any museum of natural history, priorities have to be established concerning restoration efforts for microscope slides. The decision to repair or restore a slide will largely depend on the degree of deterioration, on the value of the specimen for science, and possibly on how well represented a species is at that museum (Notton 1995; Brown & Boise 2005, 2006). Deterioration of the mounting medium requires reaction without delay if cavities or crystals have formed near or at the specimen. The specimen cannot be investigated microscopically if it is exposed to air in a cavity. The situation is utmost dramatic if crystals or cracks occur in the immediate vicinity of or even on the specimen, because the crystals and cracks will grow over time and inevitably destroy the integrity of the specimen mechanically (Figs 16G–J, 17A–F, 18A–D, F, 19A, D; Pritchard & Kruse 1982, p. 84; Neuhaus & Kegel 2015, fig. 18AC). Insect slide "specimens dry on point should be wetted with a drop of absolute alcohol, soaked free with 5% NaOH, washed in 50% alcohol and then treated as fresh material" (Mound & Pitkin 1972, p. 125). It remains questionable whether or not this is the best approach for all invertebrate material.

It may be wise to try out methods with slides of less important specimens before attempting them on type material (Gunter & Brown 2005). Older specimens do not react to conservation treatment the same way and within the same time interval as fresh material. Consequently, seemingly "worthless" old voucher specimens, even if dried-up, should not be disposed of in museum collections, because such material can still serve as training items.

The need for restoration of a microscope slide may originate from (1) a broken glass slide, (2) a specimen mounted between two coverslips with an insufficient supporting structure, and (3) problems with the mounting medium. A broken slide may be glued together or, alternatively, the specimen can be re-mounted. An insufficient supporting frame made of wood, cardboard, or plastic (Fig. 4D–I) should be replaced by a Cobb aluminum slide. If the mounting medium has deteriorated, the medium may be either strengthened without removal of the coverslip, or it may be replaced entirely by a new medium.

Restoration of microscope slides requires both general and specialized lab equipment. It is certainly helpful to provide at least one dedicated workplace just for mounting and restoring microscope slides and equip this place with the accessories listed in Table 12.

3.10.2 Restoration of broken glass slides

Generally, histological sections on a glass microscope slide are difficult to remove and re-mount on a new glass slide without significant losses. Therefore, the pieces of a broken slide with sections may only be glued together (but see below). Such a restored slide may be stabilized subsequently with a large coverslip or a 2nd glass slide below the broken glass slide (Fig. 3K) attached with neutral Canada balsam. This may impact application of the correct Köhler illumination because of the thicker glass layer towards the side of the condenser (Gill 2013, p. 319), but this should be outweighed by the advantage of being able to study the specimens at all.

For broken microscope slides, cyanoacrylates such as Technovit® 4004a or Loctite® 5332 can be used only as a temporary hold for pieces of glass, because any such adhesive will break down soon due to interaction of the adhesive with the alkaline surface of the soda-lime glass slide (Jackson 1982; Robson 1992, p. 187; Davison 2003, p. 217; Horie 2011, p. 166). A slide locally fixed with a cyanoacrylate can be subsequently glued with an epoxy resin (Jackson 1982; Robson 1992, p. 187). This procedure was described and illustrated in detail by Gütebier (2011, pp. 73–74, figs 1315) similar to a procedure suggested by Terwen (1983) for the stained glass windows of a church. It is most important to elevate the pieces of the glass slide from the underlying surface in order to avoid adhesion to this surface by the cyanoacrylate and epoxy resin (comp. Gütebier 2011, fig. 14). Epoxy resins for adhering glass (Tab. 12) have been developed for conservation (HXTAL-NYL-1 and Fynebond) or commercially (Araldite 2020) (Davison 2003, pp. 210215; Horie 2011, p. 297). Davison (2003, p. 212) reported that HXTAL-NYL-1 "takes a week to cure fully" and "the resin component of Fynebond tends to crystallize at room temperature and so has to be melted before being mixed with its hardener". Aliphatic epoxy resins like Araldite AY103/HY956, Araldite 2020, and Epotek 301-2 are more sensitive to moisture than the resins mentioned previously (Davison 2003, p. 212). Especially HXTAL-NYL-1 and Araldite AY103/HY956 reveal ageing effects like yellowing after a considerably longer time than Epotek 301-2 and a large number of other epoxy adhesives (Down 1984, 1986, 2001). Epoxy resins may deteriorate over time if the relative humidity is above 70% or if the plasticizer is lost (see chapter 3.8.7 Glyceel (= Zut, Thorne ringing compound); Horie 2011, pp. 290, 296).

Some articles describe removal of smear preparations or histological sections from a glass slide (Romeis 1948; Brown 1983; Wenger 1985; Brown & Tao 1992; Sanderson 1994). Brown & Tao (1992) soak a slide in xylene to remove the coverslip, cover the sections with Pro-Texx (from Lerner Laboratories, New Haven), and harden this ensemble in an oven overnight. The next day, the slide is warmed in tap water at 55°C for several hours until the section-Pro-Texx mount can be carefully peeled off the glass slide with a scalpel. Sections are collected floating with the section-side down on a warm water bath at 45°C, placed with the section-side down on a albumized new glass slide, blotted dry, smoothed with a small print roller, and dried in an oven at 60°C overnight. The next day, the Pro-Texx film is dissolved in xylene, and sections may be re-stained or directly re-mounted. This procedure has been developed for smear preparations, but may also work for histological sections. One limitation may be that Pro-Texx may not fully penetrate the entire thickness of the section. Romeis (1948, pp. 199–200) suggested a similar technique using "Sprimoidlack für histologische Zwecke", which is not available anymore, whereas Wenger (1985) applied Diatex Liquid Cover Glass M7638 (see also Zimmerman 1963), which does not seem to be available in 1992 anymore (Brown & Tao 1992). Diatex seems to consist of poly(methyl methacrylate) and certainly other components (for properties see chapter 3.7.16 Polyacrylates and polymethacrylates; Streble 1963). Sanderson (1994, pp. 209–210) recommended to cover sections with DPX after removal of the coverslip, let this dry, strip the sections with the DPX from the slide, mount on a new slide, and dissolve the DPX. Richardson (2014, pp. 54–57) reported the successful removal of three ca. 100 µm-thick sections from a more than 100 year old microscope slide by soaking the mount in a mixture of 10% ethanol and xylene. The thickness of the sections certainly helped in this operation. Also, the sections were possibly not glued too strongly to the glass slide anymore.

TABLE 13. Restoration steps of slides for different mounting media. Partly modified from cited references. See also text.

Example	Restoration sequence	Potential problem during restoration in certain steps	References
(A) Technique for removal, drying, deacidification, and adhesion of paper label			
paper label	(1) *Warm water in Petri dish:* soak label by immersing slide entirely (Fig. 27H) or only at its ends for several minutes to hours, (2) take up label with polyester fleece Hollytex 80 g/m², deposit on blotting cardboard 450 g/m², and remove remnants of glue by carefully wiping with absorbent purified cotton wool, (3) sandwich label between polyester fleece, blotting cardboard, and coated wooden plate on both sides, weight with several kilograms, and let dry for a couple of days, (4) deacidify label by Bookkeeper™ pump spray (provides also a buffer reserve), (5) glue label on slide with Klucel G® or store in 4-flap envelope or in Secol envelope (Fig. 27E–G).	(1): If slide is not supposed to be submerged in water, apply poultice of wet blotting paper or blotting cardboard to label (4): alternatively, (a) neutralize label for at least 30 min in Petri dish / tray with 0.15% calcium hydroxide in water, pH = 7–8.5 or (b) get label deacidified as bulk process by specialized company (e.g., Zentrum für Bucherhaltung, Preservation Academy).	Garner & Horie 1984; Pauk & Watering 1993; Neuhaus *et al.* 2012; Schhönbohm pers. com.
(B) Technique for restoration of water-soluble medium without removal of coverslip			
gum-chloral media	(1) S*tereo microscope (steps 1–5):* partly remove coverslip seal with scalpel, (2) *hotplate < 60°C:* soak slide in distilled water in Petri dish covered with watch glass and lid (for labels; Fig. 27I) for ca. 4 hours to 2–4 days until cavities in mounting medium begin to disappear, (3) take out slide of Petri dish for further processing, (4) *ambient temperature (steps 4–6):* add fresh diluted mounting medium to margin of coverslip, blotting paper on opposite side may accelerate diffusion of fresh medium, (5) repeat step (4) several times for about a week, (6) remove surplus medium with scalpel, clean with water and ethanol, and ring 2–5 times with coverslip seal on turntable, obey drying time between seal layers.	(2): (a) Coverslip seal should remain intact in parts in order to keep slide and coverslip connected, (b) coverslip may detach inadvertently, so regular control of soaking process is necessary.	Fain 1980; Neuhaus & Kegel 2015
(C) Technique for replacement of water-soluble medium			
gum-chloral media, Fluoromount G™	(1) *Stereo microscope (steps 1–4) and hotplate < 60°C:* soak slide in distilled water in Petri dish covered with watch glass and lid (for labels; Fig. 27I) for ca. 8 hours to 2–4 days until mounting medium is soft enough for slipping coverslip, (2) remove water with pipette for further processing, (3) *ambient temperature for all following steps:* carefully slide away coverslip with pair of forceps or flip over with scalpel, add drop of glycerol on specimen, and remove specimen with Irwin-loop, (4) place specimen in tiny drop of glycerol on new square coverslip with paraffin ring (Fig. 27B) and re-mount as described in (**E**).	(1): (a) Add thymol crystals to prevent growth of bacteria and fungi for immersion time of more than 2 days, (b) a neutral detergent may be added to the water (Jacinavicius *et al.* 2013), (2): (a) slide must not be submerged in plenty of water while coverslip is removed in order to avoid loss of specimen in Petri dish, (b) if the mounting medium does not soften enough within several days for safe removal of coverslip, see (**B**), (3): check whether specimen remains on slide/ lower coverslip or on top coverslip.	Mound & Pitkin 1972; Jacinavicius *et al.* 2013; Neuhaus & Kegel 2015; Walter & Krantz 2009; Neuhaus pers. obs.

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TABLE 13. (Continued)

Restoration sequence	Potential problem during restoration in certain steps	References
(D) Technique for replacement of hydrocarbon-soluble medium		
(1) *Hotplate ca. 45°C; exhaust tube connected with hood for steps 1–4:* soak slide in xylene, methyl salicylate, xylene : ethanol 1:1, or xylene + 10–20% ethanol (Canada balsam) / Euparal essence or 95% ethanol (Euparal) in Petri dish covered with watch glass and lid (for labels) for ca. 8 hours to 2–4 days until mounting medium is soft enough for slipping coverslip, (2) remove solvent with pipette or take out slide of Petri dish for further processing, (3) *stereo microscope and ambient temperature for all following steps:* carefully slide away coverslip with pair of forceps or flip over with scalpel, add drop of neutral Canada balsam/ Euparal on specimen, and remove specimen with Irwin-loop, (4) place specimen in drop of neutral Canada balsam/ Euparal on new slide/ square coverslip 24 mm x 24 mm, add circular top coverslip (Ø 15 mm), let dry for about a week at about 20°C and another few weeks to months in an *oven at about 45°C*, (5) remove surplus medium with scalpel, clean with ethanol and xylene, and ring 2–5 times with coverslip seal on turntable, obey drying time between seal layers.	(2): Slide must not be submerged in solvent while coverslip is removed in order to avoid loss of specimen in large solvent-filled Petri dish, (3): if old mounting medium does not dissolve sufficiently but allows removal of coverslip, scratch away old medium far from specimen with scalpel and cover specimen and remnants of old medium with fresh medium, which does not appear too liquid, (4): check mount during drying and add mounting medium to edge of coverslip if medium withdraws because of evaporation of volatiles.	Mound & Pitkin 1972; Moore 1979; Thatcher 1987; Huys & Boxshall 1991, p. 450; Brown & Boise 2005, 2006; Woessner 2005; Richardson 2014, pp. 17, 54–57; Neuhaus pers. obs.
(E) Technique for mounting (steps 1, 2, 6–10) and re-mounting (steps 1–10) reversible slides		
(1) *Hotplate 60–65°C:* melt paraffin for histological mounting in small Petri dish, (2) dip metal cylinder (Ø about 12 mm, thin wall) into molten paraffin and subsequently on square coverslip (24 mm x 24 mm)/ glass slide on hotplate, remove glass slide/ coverslip from hotplate and let cool (Fig. 27B), (3) *ambient temperature for steps 3–6:* hold slide/ basal coverslip, carefully lift top coverslip with scalpel and flip it over so that the side with the glycerol is on top, (4) *stereo microscope for all following steps:* check whether specimen remains on slide/ basal coverslip or on top coverslip, (5) add small drop of glycerol on specimen and remove specimen with Irwin-loop, (6) place specimen in tiny drop of glycerol on new square coverslip (24 mm x 24 mm) with ring of paraffin (Fig. 27B) and add circular top coverslip (Ø 15 mm), (7) *hotplate < 60°C:* place sandwich of 2 coverslips and specimen on hotplate, let paraffin melt and carefully manipulate top coverslip and consequently the specimen below with spinose tip of skewer stick while on hotplate, (8) *ambient temperature for steps 8–10:* remove sandwich from hotplate, check orientation of specimen under microscope with DIC, and repeat steps 5–6 if necessary, (9) mount sandwich on Cobb aluminum frame (Fig. 27C, D), (10) scratch away surplus medium with scalpel, clean with ethanol, and ring 2–5 times with coverslip seal on turntable, obey drying time between seal layers.	(1): Paraffin must be hot enough not to form a skin on the surface, but low enough not to deteriorate, (3): (a) move scalpel very slowly and with a controlled movement only in the periphery of coverslip in order not to hit specimen erroneously with scalpel, (b) top coverslip may break during lifting with scalpel, so the coverslip may have to be removed in several steps, (c) do not place glycerol-paraffin mount on hotplate at this stage, because border between glycerol and paraffin would become obscured and specimen may get covered with paraffin, (6): keep storage bottle with glycerol on hot plate, in oven, or in desiccator with silicagel to avoid uptake of humidity, (7): (a) if paraffin ring contains sufficient paraffin, the latter will keep the coverslip from crushing the specimen mounted in the glycerol, (b) if specimen gets partly covered with paraffin during mounting on hotplate, remove specimen as described, "wash" specimen 3x in 3 fresh drops of glycerol on separate slide on hotplate to melt paraffin, and re-mount	Maeseneer & d'Herde 1963; Hooper 1970, 1986a, 1986b; Hooper *et al.* 2005; Orajay 2005a, 2005b; Neuhaus pers. obs.

3.10.3 Restoration of slides—labels

In case of the necessity to restore a microscope slide or just its label and before further measures are taken, the slide should be photographed in order to document all label information (Garner & Horie 1984; Jacinavicius *et al.* 2013), and the catalogue number should be inscribed with a diamond-tipped or tungsten carbide-tipped engraving scribe. In contrast to Wilson (1971), an old label should not be disposed of but either be re-attached to the slide or be stored separately (see chapter 3.9.5 Storage of old labels). The following procedure has been suggested by a paper conservator during a project concerning wet collections at the Museum für Naturkunde Berlin (Neuhaus *et al.* 2012; Schönbohm pers. com.). The paper label may be removed in the warm (about 45°C) water bath of a Petri dish for 5–30 min by immersing the slide entirely (Fig. 27H) or only at its ends or if the slide is not supposed to be submerged in water (Tab. 13: Technique A; Garner & Horie 1984), by applying a poultice of wet blotting paper or cardboard to the label. The label can be taken up with a polyester fleece Hollytex 80 g/m² (Tab. 12), deposited on blotting cardboard 450 g/m² (Tab. 12), and remnants of glue may be removed by careful wiping with absorbent purified cotton wool (Tab. 12). The cleaned label is sandwiched by a polyester fleece, a blotting cardboard, and a coated wooden plate on both sides, weighted with several kilograms, and left to dry for a couple of days. The usage of polyester fleece as an intermediate layer is recommended, because the fleece does not adhere to the label, even if remnants of glue remain here.

Subsequently, the label may be deacidified by pump spraying it with the water-free magnesium oxide-based Bookkeeper™ (Tab. 13: Technique A; Pauk & Watering 1993) in a mixture of methoxy nonafluorobutanes and a proprietary dispersant (http://www.gmw-shop.de/datenblaetter/42215e.pdf). The magnesium oxide particles of about 1 µm diameter probably react with the ambient humidity to magnesium hydroxide, which neutralizes the existing acids in the paper and buffers the paper (Baty *et al.* 2010; http://www.ptlp.com/en). Alternatively, a label may be neutralized for at least 30 min in a Petri dish or tray with a solution of 0.15% calcium hydroxide in water, pH = 7–8.5. However, the latter application does not provide a buffer reserve on the paper in contrast to the former treatment with Bookkeeper™. Such a buffer reserve is recommended, because deterioration of the paper will continue and will result in acidic products, which accelerate degradation processes (Ahn *et al.* 2013). Bulk processing of labels (Fig. 27F) can be handled by specialized companies, in Germany these are, e.g., Zentrum für Bucherhaltung (ZFB, http://www.zfb.com/en/home) and Preservation Academy (PAL, http://preservation.academy/), both in Leipzig. Degradation processes of paper and deacidification techniques were reviewed by Baty *et al.* (2010). For adhering a label to a slide see chapter 3.9 Labels.

3.10.4 Problems with mounting medium—general considerations

Before starting any restorative efforts, the registry catalogue, database, and if necessary the original literature about the specimen in question should be consulted in order to figure out, which preparation techniques, mounting medium, and coverslip seal may have been used. In the senior author's experience, such information is not always reliable, but it represents a start. Initial conclusions may be drawn from looking at the specimen, because some mounting media show characteristic signs concerning the color, background of the medium, or deterioration artifacts (Figs 15–20), e.g., aged Canada balsam appears often yellowish to brownish (Fig. 15E); Hoyer's medium may reveal a brownish of often finely granulated background if mixed with iodine and potassium iodide; radially growing and rectangularly crystals seems to occur especially in media containing poly(vinyl alcohols) (Figs 18B–H, 19A, D, 20B–E); Permount™ shows a regular pattern of cracks (Figs 16D–J, 17A–F). One of the more advanced techniques includes taking a spectrum of the mounting medium with a Raman-microscope and comparing the result with spectra of known mounting media; this technique is currently developed in a cooperative project of the authors of this paper, which will result in a publicly available database of Raman spectra of known mounting media (Schmid *et al.* 2016). Pritchard & Kruse (1982, 1984) suggested dissolving any crystallized medium immediately with xylene. However, crystallization is very common also in water-soluble media (see above chapter 3.8 Mounting media; Tab. 6), so their approach is not recommended here if the nature of the mounting medium is unknown.

Repair of a damaged coverslip seals should be done by completely removing the seal manually and chemically and re-sealing the coverslip. It is not recommended to apply a new layer of Glyceel on top of old layers, because

"the solvent may cause the original glyceel to wrinkle" (Hooper 1986b, p. 317). Once the label of a microscope slide has been removed (Tab. 13: Technique A), the coverslip seal if existing, must be scratched away with a scalpel or needle before immersing the slide in a solvent to soften and finally dissolve the mounting medium. The coverslip seal may be softened before mechanical removal by applying water or an organic solvent, depending on the nature of the seal. For procedures how to deal with specimens mounted in water-solvable or hydrocarbon-solvable media refer to Table 13. Additional general hints are given below.

Immersion of the slide is best done in a Petri dish of appropriate size, covered with a watch glass (Fig. 27I). In this way, the curved watch glass will collect any evaporating solvent in its center and allow it to drip back into the fluid opposite to a flat lid, from which liquid will easily drip onto the hot plate at its margin. The catalogue number should be inscribed onto the slide with a diamond-tipped or tungsten carbide-tipped engraving scribe if not available already, and a new small label made of paper with the catalogue number should be placed in the solvent. The original slide label should under all circumstances remain with the slide and can be stored on top of the watch glass covered by the lid of the Petri dish (Fig. 27I) or in a second container (Brown & Boise 2005, fig. 3), so the label cannot be blown away in a hood or by any breeze caused by the sudden hand movement by lab staff. This is especially important if several slides are restored at the same time in the same area. The senior author recommends keeping movement of a Petri dish with a soaking slide to a minimum in order not to lose the specimen if the coverslip detaches inadvertently. Moseley (1943b) as well as Brown & Boise (2005, 2006) suggested cutting a glass slide with a diamond stylus into three pieces, two pieces with the labels and the central piece with the mounted specimen; the central piece of the slide was subsequently soaked in an embryo dish to dissolve the mounting medium. While this procedure allows one to use a dish with a smaller diameter of about 28 mm and therefore decreases the risk of losing a specimen, the technique becomes problematic if the mounting medium does not dissolve as intended—and this has been the case with a kinorhynch species (Neuhaus & Kegel 2015). This problem may be circumvented if one slide of a series with presumably the same mounting medium is tested for solubility before cutting slides into pieces. Any restoration procedure requires a stereo microscope with a swing-arm stand and a heavy base so a slide can be manipulated directly on the hotplate (Tab. 12). This arrangement is also necessary for making glycerol-paraffin mounts (Tab. 13).

Specimen and coverslip "should not be helped out" of the mounting medium, because the risk of breaking off parts of the animal is too high, so patience over a couple of days is necessary until they float free (Brown & Boise 2005, 2006; Woessner 2005; Jacinavicius *et al.* 2013). Pritchard & Kruse (1982, 1984) and Moore (1996) suggested flicking the coverslip off of a deteriorating mount after deep-freezing. We are not sure in how far a specimen may be damaged by this drastic procedure. Axel Christian (pers. com.) reported using hot water poured repeatedly on slides mounted with gum-chloral media. Woelke & Göke (1984, p. 212) recommended using plenty of mounting medium in order to avoid more fragile specimens being crushed by the coverslip and more rigid specimens to crack the overlying coverslip. Moore (1979) restored specimens from the middle of the 19th century mounted in Canada balsam by soaking the old mountant in xylene for up to two days, removing old balsam far from the specimen with a scalpel, and covering the specimen surrounded by remnants of old balsam with fresh Canada balsam (Moore 1979, p. 490). Garner & Horie (1984) restored slides with mosses from the late 19th century by re-mounting specimens in glycerol-gelatin, ringed with a mixture of zinc oxide and gum dammar, and subsequently with brown cement, a solution of shellac in ethanol.

Mounts with Canada balsam at the Natural History Museum in London are only restored, when a slide is broken, when phenol balsam turned black, or when a specimen needs to be macerated again for new taxonomic studies of old material (Brown & Boise 2005, 2006). Amazingly, specimens from blackened phenol balsam are re-mounted in the same kind of medium again, although the phenol seems to be responsible for the deterioration of the mounting medium (Stroyan in Eastop 1985; Brown 1997; Brown & Boise 2005, 2006). In a similar approach, Pritchard & Kruse (1982, p. 78) dissolved the blackened mounting medium with xylene, and the specimen was soaked in beechwood creosote, transferred to 70% ethanol, soaked in 5% hydrochloric acid in 70% ethanol, dehydrated, and mounted in Canada balsam.

Cavities in a mounting medium reaching the margin of a coverslip may be filled by repeatedly positioning a small drop of fresh medium with a needle at the margin of the coverslip over a period of several days (Woessner 2005). This author also suggests to slightly warm the slide for a short time. Heating at a higher temperature may lead to a softening of the mounting medium, and a bubble may be squeezed out by carefully pressing the coverslip with a needle (Woessner 2005). Too much heat will result in the development of more cavities, darkening of the

mounting medium, and damage to the specimen. Therefore, heating a slide requires both patience and experience. For a technique for filling cavities more centrally under a coverslip see Table 13: Technique B.

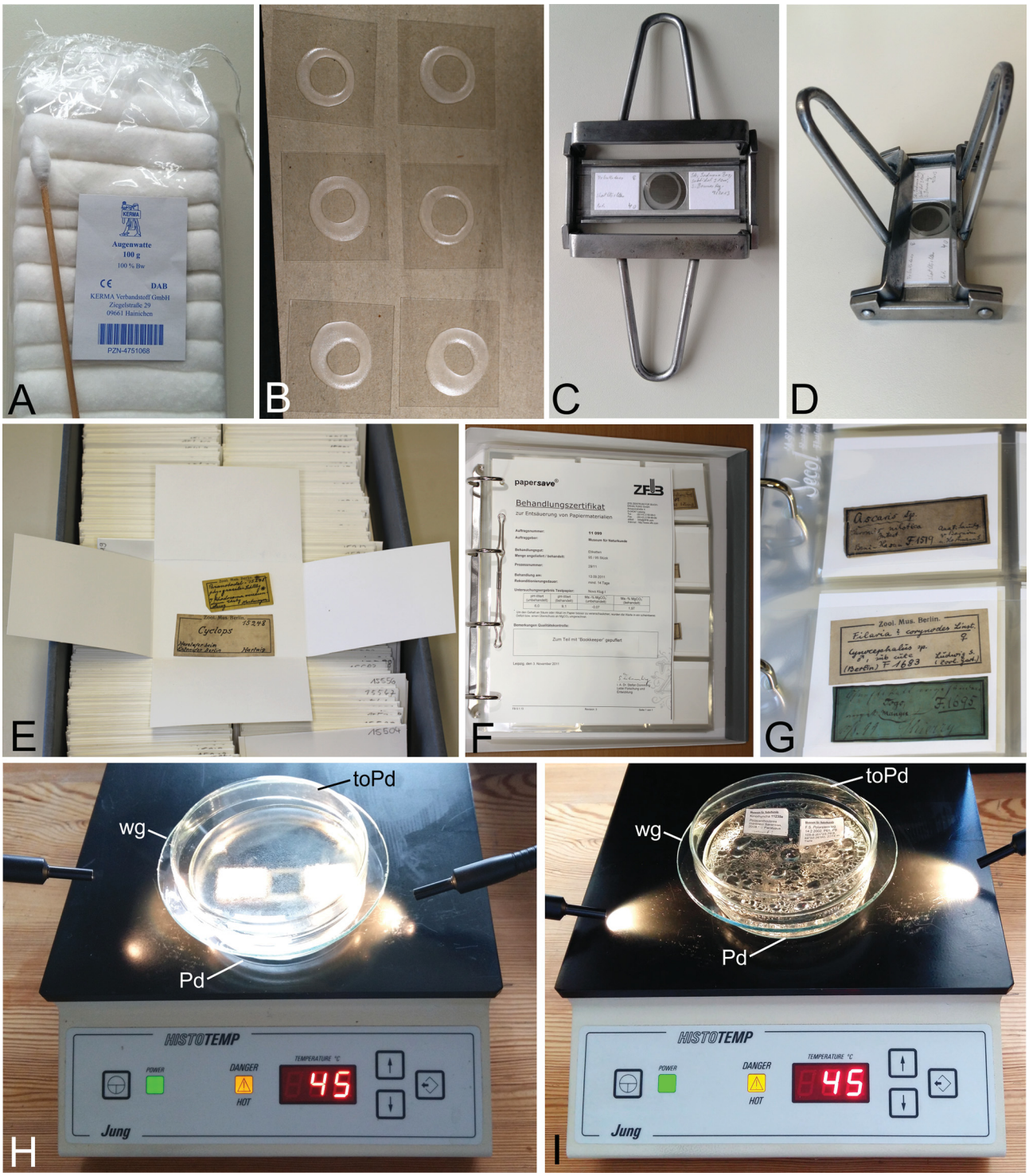

FIGURE 27. A. Absorbent purified cotton wool wrapped on skewer. **B.** Coverslips 24 mm x 24 mm with paraffin rings ready for mounting each specimen in a drop of glycerol on a Cobb aluminum slide. **C, D.** Slide mounting device for Cobb aluminum slides. **E.** Storage of labels in 4-flap paper envelopes filed in cardboard boxes provided by Klug Conservation. **F, G.** Storage of labels in Secol polyester slide and negative film preserver sheets housed in a custom-made cardboard file system made by Klug Conservation. Note certificate of deacidification with Bookkeeper™ in front of the labels (**F**) and sheet of paper behind labels in polyester envelope (**G**). **H, I.** Restoration of a microscope slide by soaking the slide in distilled water on a hot plate in the lower part of a Petri dish (Pd) covered with a watch glass (wg) before (**H**) and after removal of labels and their storage under the top of the Petri dish (**I**, toPd). Note drops of water dripping back into lower Petri dish (**I**).

3.10.5 Problems with liquid mounting media—re-hydration of specimens

A solidifying mounting medium may develop cavities at some stage, and the specimen will be exposed to air (Figs 3D, 18A–C, 19A, D, E, 20B–D). Usually, such a specimen maintains its structure with the help of remnants of the mounting medium. Therefore, the medium may be dissolved with water or an organic solvent like xylene, no extra care is necessary for the specimen as long as the correct solvent is used (but for a case report see chapter 3.7.15 Permount™). However, if a specimen has dried-up on a slide because of evaporation of a fluid mountant, it will collapse like a mummified specimen (Prats-Muñoz *et al.* 2015, p. 285) or a specimen from a wet collection having lost its preservation fluid, because there is no supporting medium. Consequently, a dried-up specimen from a microscope fluid mount has to be re-hydrated and treated similar to a dried-up specimen from a wet collection.

Numerous recipes about the re-hydration of biological material can be deduced from zoological, forensic, pathological, and paleopathological studies back to the early 20th century (Tabs 14, 15). Whereas almost all papers refer to medium- to large-sized mummified specimens or to such specimens from wet collections (Tabs 14, 15), a single study has been found specifically about the re-hydration of animal and plant tissues on microscope slides (Moore 1979). This author reported restoring slides from the middle of the 19th century each with a chamber for a fluid-preserved specimen including re-hydration of specimens with 1% trisodium phosphate in some cases. For fixation of specimen to be re-mounted he used Steedman's fixative and for the preservation fluid in the cell Steedman's post-fixation preservative containing propylene phenoxetol and propylene glycol (Tabs 14, 15; Steedman 1976b, pp. 179–181). Propylene glycol increases the viscosity of a fluid, softens the specimen, slightly raises the refractive index, and inhibits growth of mold. Propylene phenoxetol is an anti-oxidant, bactericidal, and fungicidal (Steedman 1976b, p. 179–180). Coverslips were sealed with bitumen in toluene (Moore 1979). One reviewer of this manuscript remarked that specimens originally fixed in formaldehyde and then stored for at least 10 years in Steedman's preservative suffered catastrophic loss of tissue integrity, when examined histologically.

Generally, a re-hydration solution should possess the following properties as stated by Prats-Muñoz *et al.* (2015, pp. 284–285): "An optimal rehydration fluid diffuses quickly into the tissue, which allows it to stabilize its structure. Therefore, the rehydration solution should contain emulsifying and tensoactive agents, a preservative that inhibits bacterial growth and a rehydrating agent (Mekota and Vermehren 2005); it is important to adapt the solution to the conditions under which preservation occurred." A large variety of "emulsifying and tensoactive agents" have been used like Aerosol® OT, Antiformin, Biz laundry detergent, the fabric softener Unilever Comfort®, Contrad® 70, Decon® 90, dimethyl sulfoxide, dioctyl sodium sulfosuccinate, Drano-Max, Fit, Klean-Strip® TSP substitute, Liquid Plumber Buildup Remover, Multi-Terge™, propylene glycol, sodium carbonate, sodium sulphate, trisodium phosphate, but also macerating agents like acetic acid, Contrad® 70, Decon® 90, potassium or sodium hydroxide, lactic acid, sodium citrate, and sodium hypochlorite, as well as buffers, normal saline, or disodium ethylene diamine tetraacetic acid (Tab. 15). Some authors suggested immersion of specimens into cold, warm, or hot ethanol solutions (Levi 1966; Ellis 1981). Others apply vacuum (Cunningham 1969; Jeppesen 1988; Carter 1998), heat from microwave (Prats-Muñoz *et al.* 2015), or heat from another source (Van Cleave & Ross 1947a, 1947b; Cunningham 1969; Banks & Williams 1972; Pritchard & Kruse 1984; Carter 1998). Concentrations of agents and length of time of application vary considerably, depending on which taxonomic group is treated, on the size of a specimen, and on the kind of tissue (Tab. 15; Prats-Muñoz *et al.* 2015). The outcome of re-hydration procedures seems to be quite variable (Tab. 15). Maceration of specimens may be disastrous, especially after too long exposure to some re-hydration solutions. Anti-bacterial and anti-fungal precautions should be taken but have not in every case, e.g., by addition of thymol crystals or another preservative to the soaking solution. No miracles should be expected from re-hydrating specimens. Although the outer shape of a specimen may be restored more or less completely, the cellular morphology will have suffered to a various degree (Tab. 15). Great care, tests, and frequent controls should be used when re-hydrating a specimen, especially if soft-bodied invertebrates of less than 1 mm body size on microscope slides have to be treated. The first major problem represents the identification of such dried-up tiny specimen on a slide before starting the re-hydration procedure (Neuhaus pers. obs.).

TABLE 14. Ingredients of re-hydrating and selected subsequent fixation solutions.

Chemical agent	Ingredients	Source
Aerosol® OT	sodium bis(2-ethylhexyl) sulfosuccinate	Sigma-Aldrich[1]
Agepon (= Wac wetting agent; used in photographic film processing before drying the film)	4.9% sodium alkylbenzene sulfonate (C 10–13), 2.3% sodium sulfonate (C 10–18-alkane)	A & O[2]
Antiformin	7.5% sodium hydroxide (NaOH) and 5.3% sodium hypochlorite (NaOCl) in water	Böck 1989, p. 79
Biz laundry detergent	probably not available in original composition anymore	
Bouin' solution	15: 15: 1 picric acid: 30% formaldehyde: glacial acetic acid	Mekota & Vermehren 2005
Carosafe® concentrate (dilute 1:9)	91% propylene glycol, 6% 2-amino-2-ethyl-1,3-propanediol, 3% 2-phenoxy ethanol	Carolina[3]
Unilever Comfort® fabric softener	di-hardened tallow dimethyl ammonium chlorides, colorants, perfume, preservatives	Turner & Holtom 1981
Contrad® 70	≤ 5% KOH, *n*-dodecylbenzene sulfonic acid, ethoxylated propoxylated alcohols (C 10–12), 6–10% trisodium citrate dihydrate, triethanolamine	VWR[4]
Decon® 90	0.5–2% KOH, anionic & non-ionic surfactants, stabilising agents, non-phosphate detergent builders	Decon[5]
modified Farmer's solution	3:1 ethanol: glacial acetic acid	Prats-Muñoz *et al.* 2015
Fit	sodium dodecylpoly(oxyethylen) sulfate, cocamidopropyl betain, sodium chloride, parfum, amylase, 2-bromo-2-nitropropane-1,3-diol, benzisothiazolinone, methylisothiazolinone, colorant	Fit[6]
10% formal saline	4% formaldehyde and 0.9% sodium chloride in water	
Harleco® Multi-Terge™	nitrilotriacetic acid trisodium salt, emulsifier-K-30, sodium hydroxide, -dodecyl-omega-hydroxypoly(oxyethylene)	VWR[7]
Klean-Strip® TSP substitute	< 5% 2-(2-butoxyethoxy)ethanol	Barr[8]
Metaflow	10–25% propylene glycol, 1–10% dimethyl sulfoxide, 1–10% trisodium ethylenediaminetetraacetic acid (EDTA)	Dodge[9]
normal saline	0.9% sodium chloride in water	
Restorative	polyoxypropylene glycol, propylene glycol, polyethylene glycol, trisodium ethylenediaminetetraacetic acid (EDTA), isopropanol, ethoxylated Lanolin	Dodge[9]

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TABLE 14. (Continued)

Chemical agent	Ingredients	Source
Sandison's solution	5:3:2 1% formaldehyde: 96% ethanol: 5% sodium carbonate in water	Sandison 1966; Prats-Muñoz *et al.* 2015
modified Schaffer's solution	2:1 80% ethanol: 36% formaldehyde	Mekota & Vermehren 2005
modified Schaffer's solution	9:1 80–96% ethanol: 10% formaldehyde	Prats-Muñoz *et al.* 2015
Steedman's fixative	89:5:5:1 water: propylene glycol: 40% formaldehyde: propylene phenoxetol	Moore 1979; also Steedman 1976b
Steedman's post-fixation preservative	89:10:1 water: propylene glycol: propylene phenoxetol	Moore 1979; also Steedman 1976b
WARDsafe™ concentrate (dilute 1:9)	50% water, 28.7% methanol, 14.4% propylene glycol, 4.8% phenoxy ethanol, 1.6% glutaraldehyde, 0.3% sodium lauryl sulfate, 0.2% amyl acetate	Ward Science[10]

[1] Sigma-Aldrich: Available from: http://www.sigmaaldrich.com/catalog/product/aldrich/323586?lang=de&region=DE (accessed 27 July 2016).
[2] A & O: Available from: http://www.maco-photo.de/files/images/Agfa_Agepon_eng.pdf (accessed 21 July 2016).
[3] Carolina: Available from: http://www.carolina.com/pdf/msds/carosafeaghs.pdf (accessed 26 July 2016).
[4] VWR: Available from: https://us.vwr.com/store/product/9231129/contrad-70-liquid-detergent-decon-labs (accessed 21 July 2016).
[5] Decon: Available from: http://www.decon.co.uk/english/decon90.asp (accessed 21 July 2016).
[6] Fit: Available from: http://www.fit.de/fit/spuelen/mit-der-hand/fit-spuelmittel-original/ (accessed 28July 2016).
[7] VWR: Available from: http://us.vwr.com/assetsvc/asset/en_US/id/14031159/contents (accessed 28 July 2016).
[8] Barr: Available from: http://www.kleanstrip.com/msds-cpsia (accessed 28 July 2016).
[9] Dodge: Available from: https://shop.dodgeco.com/SDS.aspx (accessed 24 February 2017).
[10]Ward Science: https://www.wardsci.com/store/product/8892316/wardsafe (accessed 26 July 2016).

TABLE 15. Methods for re-hydrating dried-up biological samples.

Method	Taxon applied to	Remarks	Source
soak in (a) 5:3:2 water: ethanol: 5% sodium carbonate (Na_2CO_3) in water or in (b) 1% formaldehyde and 5% sodium carbonate until tissue is pliable, transfer to increasing amount of ethanol	human tissues, 3,100 – 3,500 years dry	- principal recipe first published 1909 and then in more details 1911, book of Ruffer (1921) contains several reprinted articles, *inter alia* that from 1911	Ruffer 1921, p. 64
soak large specimen in 1% sodium hydroxide in 33% ethanol for 7–14 days, rinse with running tap water for 12 hours, fix in 4% formaldehyde for 7 days, dehydrate in 90% ethanol	human tissues, 150–200 years dry		Gillman 1934
soak in cold or 35°C warm 0.250.5% trisodium phosphate (Na_3PO_4•12 H_2O) in water for 1–48 hours, rinse with water, stain with hematoxylin or borax carmine or transfer to 80% ethanol	Acanthocephala, Nematoda, Hirudinea, Arthropoda	- specimen may become too flaccid, - potassium hydroxide damages tissue	Van Cleave & Ross 1947a, 1947b
boil in 85% ethanol for a short time (subsequent cooling to room temperature removes air bubbles)	museum specimens	- "Using NaOH and KOH solution swells and warps softer parts and is not recommended."	Levi 1966, p. 186
soak in 5:3:2 1% formaldehyde: 96% ethanol: 5% sodium carbonate in water until specimen is pliable, rinse with 10% formal saline, dehydrate, embed	tissue for histology; human tisues, several 1,000 years dry	- re-hydration complete within ≤ 1 day	Sandison 1966
soak in 50% ethylene glycol in water for 12–24 hours, transfer to 50%/ 70% ethanol	Decapoda	- ethylene glycol harmful for humans (Steedman 1976b; Marhue 1983: replacement by propylene glycol)	Thompson *et al.* 1966
soak in 2.5–3.3% Aerosol® OT in water for at least 5 hours, rinse with water	herbarium tissues	- Aerosol® OT is a surfactant	Ayensu 1967
soak in 3% potassium hydroxide in water at 75°C for 20–30 min, rinse with water for 1–2 hours, fix in 40:15:1 50% ethanol, formaldehyde, and propionic acid for 1 day, dehydrate, embed	herbarium tissues	- apply vacuum or alternatively heat and cool sample during processing to remove air bubbles, - re-hydration with good results even with material dry for 100 years	Cunningham 1969
soak in an alkaline detergent (Decon® 90 = Contrad® 70, Multi-Terge™) in water for 12 hours, heat to 100°C for 1–5 min if specimen is not re-inflated, wash in water twice	Hexapoda	- Decon® 90 is a surface active cleaning agent for laboratory applications, - removes also wax and macerates specimen, - Decon 90 = Contrad® 70: see Marhue (1983), - Multi-Terge™: suggestion by Stehr (1987)	Banks & Williams 1972
soak in 5:3:2 water: 96% ethanol: 5% sodium carbonate in water, transfer to increasing amount of ethanol over 3 days, embed	human tissues, experimentally dried	- re-hydration complete within ≤ 7 days, - result of re-hydration depends on proper initial fixation of tissue	Zimmerman 1972, 1976

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TABLE 15. (Continued)

Method	Taxon applied to	Remarks	Source
soak in warm 1% trisodium phosphate in water, rinse with water, fix in Steedman's fixative, preserve in Steedman's post-fixation preservative containing propylene phenoxetol and propylene glycol (Steedman 1976b: pp. 179–181), mount as wet slide	animal and plant tissues on slides	- brush away white precipitations on surface, - "properties of fixative: pH 6.8–7.0, non-hardening of tissue, non-shrinking of tissue, non-flammable, slight swelling of tissue" - specimens fixed in formaldehyde and stored for >10 years in Steedman's preservative suffer from loss of tissue integrity, when examined histologically	Moore 1979; one reviewer
soak in 80% ethanol, with or without heating to boiling, wash in water, relax in (a) 1.2% sodium chloride in water for 4–24 hours, (b) 2% trisodium phosphate in water for 4–6 hours, (c) 0.5% formaldehyde in water for 4–12 hours, (d) 5% sodium sulphate in water for 4–12 hours, (e) 1:1 2% citric acid: 20% sodium citrate for ≤ 4 hours, or (f) 3:5:2 90% ethanol: 0.5% formaldehyde: 5% sodium citrate for 1–24 hours (= Sandison's technique), wash in water, store in 80% ethanol	Isopoda	- applied to Linnaeus' collection, which was stored dry for about 115 years, - (b) specimen may become too flaccid and precipitations may develop on specimen, - (e) specimen may become too flaccid, - (f) variable results, - erroneously cites sodium citrate as ingredient of Sandison's technique (Sandison used sodium carbonate instead, see Sandison 1966)	Ellis 1981
soak in 0.2% fabric softener Unilever Comfort® in normal saline for 12 hours, fix in formaldehyde in saline, dehydrate, and embed in paraffin	human tissue, 2,600–4,100 years dry	- tissue treated with softener is softer for sectioning than tissue treated with Ruffer's or Sandison's solution, - softener does not macerate specimen on prolonged exposure	Turner & Holtom 1981
soak in 33% manual dish washing agent Fit in water, rinse with 20–30% ethanol, transfer to 75% ethanol	Hexapoda	- rinsing with water leads to additional swelling of specimens and to rupture of body wall opposite to rinsing with 20–30% ethanol, - soaking in 0.25–0.5% trisodium phosphate not satisfying	Joost 1982
(a) soak in 50% propylene glycol in water for 12-24 hours, transfer to 50%/ 70% ethanol or in (b) 0.5% trisodium phosphate for 2 hours, transfer to 75% ethanol with 2% propylene glycol for storage	Decapoda	- ethylene glycol used by Thompson *et al.* (1966) is harmful for humans, replaced here by propylene glycol (= 1,2-propanediol) - also cites recipe from Banks & Williams 1972	Marhue 1983
soak in cold or 35°C warm 0.25–0.5% trisodium phosphate	helminths		Pritchard & Kruse 1984
soak in warm 3–5% KOH in water for several days, rinse with water, neutralize with 5–10% acetic acid, transfer to 25%/ 50%/ 70% ethanol	immature insects	- Multi-Terge™ may replace Decon 90, - also cites recipes from Van Cleave & Ross 1947a; Thompson *et al.* 1966; Banks & Williams 1972; Marhue 1983	Stehr 1987, p. 15
soak in warm 50–80% lactic acid for 2 days in water, transfer to 70% ethanol			
soak in 1:1 Metaflow:Restorative for hours to days, transfer to water	human tissue		Haglund 1988

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TABLE 15. (Continued)

Method	Taxon applied to	Remarks	Source
(a) soak in 3% Decon® 90 or in (b) 2% dioctyl sodium sulfosuccinate in water alternating between vacuum and environmental pressure 4–8 times during 1–4 days (start with low pressure difference for a short time, increase vacuum each time), rinse in water, transfer to preservative	zooplankton, Polychaeta, Hirudinea, Crustacea, Chaetognatha, Echinodermata	- (a) 100%/ 3% Decon® 90: pH = 13.7/ 11.8, - (b) 100%/ 2% dioctyl sodium sulfosuccinate: pH = 7.7/ 7.4, - less recommendable: soaking in water (fair results), artificial seawater (poor results), 5% ethylene glycol (also harmful to humans; poor results), 0.25% trisodium phosphate (slight maceration of specimens; poor results), 80% ethanol (unacceptable results), - vacuum is applied to remove air bubbles	Jeppesen 1988
(a) soak in 20% Antiformin in water for 1 day or in (b) concentrated or diluted (≤ 80%) dimethyl sulfoxide (= DMSO) for 1 day	dried-up specimens for histology		Böck 1989, p. 79
soak in 40% lactic acid at 60°C for ca. 5 hours, rinse for 1 hour with running tap water, rinse with distilled water, transfer to 70% ethanol or formaldehyde	Crustacea, plankton		Geptner & Mikhailov 1989
(a) soak in 2–5% sodium tetraborate in water, (b) 1–2% potassium or sodium hydroxide in water (for invertebrates), (c) 1–14% trisodium phosphate in water (for invertebrates), (d) 5% sodium sulphate in water (for invertebrates), (e) 2–10% sodium acetate in water (for Arthropoda), (f) 1–3% trichloroacetic acid in water (for Arthropoda), (g) 2% sodium carbonate and 0.5% formaldehyde in water, (h) 1–2% saline in water, (i) 1: 1 glycerol: 10% acetic acid in water, (j) 30% ethanol in water, (k) 1% formaldehyde in water, (l) 2% citric acid and 20% sodium citrate in water, (m) clove oil (for naturally desiccated material), or in (n) 5: 3: 2 1% formaldehyde: 90% ethanol: 5% sodium carbonate	specimens in wet collections and mummified tissue		Harris 1990
soak in 10% acetic acid in water for 7–14 days (invertebrates: 3–7 days), (vertebrates only: in 5% acetic acid for 7–14 days), in 0.5% Biz laundry detergent or in 0.75–1% trisodium phosphate for 3–7 days, rinse with water for 1–8 hours, (optional: fix in 4% formaldehyde for 1–4 days), transfer to 35%/ 50% isopropanol or 35%/ 50%/ 70% ethanol for 1 day for each step	Coelenterata, Cestodes, Polychaeta, Mollusca, Echinodermata, "Pisces", Amphibia, algae	- acetic acid: pH = 3.0–3.25, - frequently check calcareous specimens, - not recommended to use chlorine-free bleaches and laundry soaps with enzymes, which destroy all soft tissue, - larger specimens respond better than smaller, - occasionally color change	Vogt 1991

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TABLE 15. (Continued)

Method	Taxon applied to	Remarks	Source
(a) soak in 1–3% potassium hydroxide, or in (b) 1–3% lactic acid	Arthropoda	- also cites recipes from Van Cleave & Ross 1947a; Joost 1982; Geptner & Mikhailov 1989	Piechocki & Händel 1996
(a) soak in 5% Decon® 90 in water, (b) warmed glycerol (small specimens only), or in (c) 2–4% trisodium phosphate in water for about 16 hours and apply vacuum to get rid of air bubbles, rinse with water for 30 min (omit this step for spiders to avoid rupture of the abdomen), transfer to 20%/ 40%/ 60% ethanol with 2–4% propylene glycol	Oligochaeta, Arthropoda	- best results with Decon® 90 but not successful for every specimen, - also cites recipes from Van Cleave & Ross 1947a; Marhue 1983; Jeppesen 1988	Carter 1998
soak in 10% enzymatic drain cleaner-build up remover like Drano-Max or Liquid Plumber Buildup Remover fo at least 3 weeks	"Pisces", Amphibia	- pH = 6.5, - results with vacuum chamber not consistent and not satisfying	Vogt 1998
soak in (a) 2% Decon® 90 or in (b) 2% trisodium phosphate in water for 17 hours, transfer to 80% ethanol	Arachnida, Myriapoda	- (a, b) leaching of color, body breakdown; - (b) flaking of cuticle, precipitations on specimen	Beccaloni 2001
soak in buffered water (1 buffer tablet from Micro Essential Laboratory per 100 ml water)	Mollusca, "Pisces", Amphibia	- pH = 10, - re-hydration influenced by preservation history: best results with specimens preserved in 70% ethanol or in a glycol-based agent (Carosafe®, WARDsafe™), poor results with specimens preserved in ≥ 50% isopropanol	Vogt 2001
(a) soak in inactivated human blood serum at 4°C, (b) 0.9% sodium chloride, (c) 70 ml 70% ethanol, 30 ml glycerol, and 1 g dithionite, (d) 1: 1 glycerol: 10% acetic acid, (e) 5% DMSO in Tris buffer, pH = 7.6, (f) 2% sodium carbonate in water, (g) 15% glucose in water, (h) 95: 5 2% formaldehyde: Brij® in water, (i) 1% potassium hydroxide, (j) 4: 1 inactivated human blood serum at 4°C: 5% sodium carbonate, (k) 5: 3: 2 water: 15% saccharose in water: 2% sodium carbonate, or in (l) 8: 2 0.2% fabric softener Unilever Comfort® in 5% sodium carbonate in water: 4% formaldehyde	human tissues (0.5 cm^3), 3,100–3,560 years dry	- also cite recipes from Ruffer (1921) and Sandison (1966), - optimal results for human skin: re-hydration with Ruffer's solution containing ethanol (Ruffer 1921) or with methods (e) or (l) followed by fixation with 4% formaldehyde, or modified Schaffer's solution (Tab. 14), or Bouin's solution (Tab. 14), - optimal results for human meniscus: re-hydration with Ruffer's solution containing formaldehyde (Ruffer 1921) followed by fixation with 4% formaldehyde, or re-hydration with method (l) followed by fixation with modified Schaffer's solution (Tab. 14), - optimal results for human placenta: re-hydration with Sandison's solution (Sandison 1966) or with method (l) followed by fixation with 4% formaldehyde, - poor results with methods (f) and (g), - disastrous results with method (i)	Mekota & Vermehren 2005

... ...continued on the next page

TABLE 15. (Continued)

Method	Taxon applied to	Remarks	Source
soak in 20% dimethyl sulfoxide (= DMSO), 0.25 M disodium ethylene diamine tetraacetic acid (= disodium EDTA), and saturated sodium chloride for 1–3 days, rinsed with water for 5–60 min, fix in AFA (= acetic acid, formaldehyde, ethanol)	Acanthocephala, Nematoda	- purpose of study is re-hydration of specimens preserved in 95% ethanol for molecular studies, - not all characters restored in Acanthocephala, - no detailed morphological characters shown	Naem *et al.* 2010
(a) soak in 5% trisodium phosphate, (b) Klean-Strip® TSP substitute with 1–5% sodium silicate as active ingredient, or in (c) 80% ethanol	larval Diptera	- specimens differ 1–19% in length and 27–95% in weight after re-hydration from initial length and weight, -"amount of restoration depends on the initial preservation method, fly species, and potentially on the length of time that specimens were preserved"	Sanford *et al.* 2011
(a) soak in 2% Decon® 90 or in (b) 5% trisodium phosphate plus 5% Agepon in water for 5.5–16 days, rinse with water, transfer to 80% ethanol	Arachnida, Myriapoda	- (a) 20% of specimens: body breakdown; - (b) 55% of specimens: leaching of fat, body breakdown, precipitations on specimen; - application of soaking solution at 50°C avoids deterioration and reduces exposure time	Beccaloni 2012
soak in 5:3:2 water: 96% ethanol: 5% sodium carbonate in water for 30 min–2 days, heat for 15 sec with microwave, fix in 9:1 80–96% ethanol: 10% formaldehyde (= modified Schaffer's solution) for 1 day, dehydrate, embed	human tissues (8-10 mm), 800–5,300 years dry	- optimal results for desiccated specimens with Ruffer's solution (Ruffer 1921), - heating accelerates fixation, prevents excessive fragmentation of specimens during re-hydration, and improves staining by coagulation of proteins in desiccated specimens, - excessive exposure to re-hydrating solution causes disintegration of tissues, - less recommendable for re-hydration of desiccated specimens but preferable for saponified tissue: Sandison's solution, - less recommendable for fixation: 4% paraformaldehyde (causes hardening of tissue) and Farmer's solution (causes excessive hardening of tissue)	Prats-Muñoz *et al.* 2015
soak 'sediment' and mites in 5 ml 70% ethanol, 3 ml 5% acetic acid, and 10 drops of diethyl ether, heat for 424 hours to 75°C; heat mite in lactic acid for several hours at 95°C (or up to 18 hours at 100–110°C); mount specimen	phytophagous Acari	- 'sediment' contained mites which were originally fixed in picric acid, hydrochloric acid, ethanol formaldehyde (now probably polymerized), or mixtures thereof; phenolic components were added to prevent microbial growth	Chetverikov *et al.* 2016

3.10.6 Problems with water-soluble mounting media

For specimens in a water-soluble mounting medium like Hoyer's mounting medium and Fluoromount G™, a straightforward approach is recommended (Tab. 13: Technique C). The senior author chooses distilled water at a temperature between 45–60°C, which is considerably higher than room temperature, in order to get a quicker dissolution of the mounting medium and to avoid growth of fungi and bacteria. The specimens are re-mounted on a Cobb aluminum slide allowing microscope observations from both sides (Hooper 1970, 1986a; Westheide & Purschke 1988). The upper coverslip is sealed with Glyceel prepared according to the recipe of Bates (1997). Koomen & Vaupel Klein (1995, p. 433) immersed microscope slides in 70% ethanol overnight to dissolve both the coverslip seal nail varnish and Berlese's mounting medium.

Water-soluble mounting media like Berlese's medium (e.g., for slides with insects) may be soaked for several days at room temperature in 30% ethanol, which inhibits growth of fungi (Brown & Boise 2005, 2006). A subsequent bath in 10% potassium hydroxide for 5–30 min would often dissolve remnants of the mounting medium; otherwise, a soak in warm 10% potassium hydroxide or in warm acetone may have to follow for some mounting media (Brown & Boise 2005, 2006; Gunter & Brown 2005). Remnants of dirt or mounting medium may also be removed from a specimen like a mite by adding a few drops of sodium hypochlorite to the soaking water in a Petri dish and letting it work for 1–3 min (Jacinavicius *et al.* 2013). Before applying these methods originally developed for arthropods to other invertebrates, careful tests should be done to make sure the specimens are not damaged by the chemicals. Morse (1992) suggested re-hydration of slides in a humidity chamber containing silica gel saturated with distilled water and a very small amount of ethanol to avoid growth of mold; too much ethanol may result in reaction with the mounting medium as can be confirmed for different gum-chloral media, which turn cloudy if exposed to ethanol (Jeppson *et al.* 1975, p. 388; Bink 1979; Garner & Horie 1984; Neuhaus pers. obs.).

If the coverslip does not detach from a water-soluble medium within two days, the senior author suggests stopping soaking, adding fresh mounting medium repeatedly to the edge of the coverslip, letting this dry for a couple of days, and finally ringing the coverslip with Glyceel (Tab. 13: Technique B; Brown 1997, p. 9). This procedure closed the cavities in the mounting medium widely (supposedly Hoyer's mounting medium) in specimens of the kinorhynch *Cateria styx* Gerlach, 1956 from Chile and allowed microscopic studies again but did not dissolve all crystals (Neuhaus & Kegel 2015, comp. fig. 18D with fig. 18F). And, as Neuhaus & Kegel (2015) suspect, the water may "at some stage evaporate again and the mounting medium may reach a similar condition as before" revealing cavities and precipitations.

In another case, the kinorhynch species *Pycnophyes frequens* Blake, 1930, the mounting medium was deteriorating and represented possibly CMC-10, Hoyer's mounting medium, or Permount™ (soluble in toluene), according to references from that author at that time (Higgins 1961, 1964) but not stated in the original publication (Higgins 1965). The medium dissolved in water within a few days in one slide, where cracks and crystals had reached the specimen indicating either Hoyer's or CMC-10. In a second specimen, where cracks appeared only in the periphery, the medium without cracks turned yellowish-milky but did not dissolve entirely. Pieces of original mounting medium remained with the specimens in both specimens and could not be removed; this is only seen at higher magnifications with a microscope. Specimens were re-mounted as glycerol-paraffin slides; the specimens did not soften in glycerol as they usually do and could also not be manipulated to a flatter size (Neuhaus pers. obs.). The latter two examples indicate the importance of being able to identify a mounting medium properly with a non-destructive technique.

3.10.7 Problems with hydrocarbon-soluble mounting media

For specimens in a mounting medium soluble in an organic solvent like Canada balsam, an approach analogous to the one described for water-based media above seems recommendable (Tab. 13: Technique D). Thatcher (1987) reported soaking a microscope slide with Canada balsam either in xylene or in methyl salicylate. Richardson (2014, p. 55) mentioned that soaking a slide in toluene or xylene alone may result in a "milky sticky semi-insoluble substance" and suggests to add 10–20% ethanol to either toluene or xylene; re-mounting should be done via rinsing in pure toluene or xylene. Brunner & Blueford (1986) used an alternative approach for re-mounting fossil Radiolaria and heated the slides with Canada balsam in a microwave until it melted. This procedure did not yield satisfying results with fossil graptolites mounted in the same medium, but led to darkening of the medium and the

formation of vesicles (Allington-Jones 2008). Heating of Canada balsam until it melts at 80°C (Lillie *et al.* 1953, p. 59) cannot be recommended for recent biological material, because the heat applied to melt the medium may harm the specimen mounted. For our own experience with Permount™ see chapter 3.7.15 Permount™.

3.10.8 Overly macerated specimens

It is known that specimens may be intensely macerated, e.g., by gum-chloral media, so that they cannot be recognized even by a microscope equipped with differential interference contrast (Kinorhyncha: Neuhaus & Kegel 2015). Similar problems occur on slides of Acari (Amrine & Manson 1996; Lillo *et al.* 2010). The latter authors suggest soaking the slide in water on a hotplate, adding a drop of iodine stain preferably with the original mounting medium, and blotting the stain with absorbent paper from the other side of the coverslip so the stain migrates over the specimen. The authors also state: "This technique may not work with very old slides, difficult media such as polyvinyl alcohol, or overly faded specimens; but if the slide is not usable, this technique is worth a try." (Lillo *et al.* 2010, p. 294). Staining may also be done alternatively with dyes like chlorazol black, eosin, light green, and orange G (Knudsen 1966, p. 274), because these components are commonly used for staining small invertebrates on slides (see also chapter 3.1.4 Staining). Delicate chitinous structures may be stained with phenol-fuchsin (Martin 1978, p. 103).

For microscope- and software-based contrasting methods see chapter 3.12.1 Microscope equipment. However, these methods may not work if the specimen is macerated excessively.

3.11 Alternatives to microscope slides

Considering the problems with mounting media for microscope slides, one might think of alternatives to store minute valuable specimens. In arthropod collections, it is common practice to keep genitalia in small glass or plastic tubes filled with glycerol or clove oil and sealed with a cork or silicone rubber stopper; this ensemble is then attached to the needle with the insect and the labels (Barr 1973; Robinson 1976; Martin 1978, pp. 103–104; Brown 1997; Jäger pers. com.). Obviously, this practice is time-saving in terms of providing specimens on loan to other scientists, and loans do consume a lot of the working time of staff in insect collections. However, it is foreseeable that plastic tubes as well as cork and silicone rubber stopper will inevitably deteriorate over time and must be replaced, requiring staff working time to do so and risking loss of the specimen's genitalia by improper handling in the collection, during transport, and by the scientist requesting the loan (Robinson 1976). Also, the vials may dry out because of leakage or evaporation of the storage liquid. Recovering the specimens from the vials and keeping track of many temporary microscope slides at the same time is both time-consuming, tedious, and erratic (Barr 1973; Robinson 1976; Brown 1997).

Valuable specimens could also be stored in a small tube contained in a larger jar in the wet collection. About 2% glycerol may be added in order to prevent complete drying out of specimens if the ethanol should evaporate; nematodes seem to last at least 46 years in this condition (Hooper 1986b). The main drawbacks of this kind of storage are the "excessive direct handling and manipulating of the specimen" for the recovery of the minute specimen from the tube for re-examination, making a proper temporary slide mount without damaging or losing the specimen, and recovering the specimen from the slide again (Martin 1978, pp. 107–108). Also, there is a high risk that a temporary mount dries out if "temporary" turns out to last much longer than expected. For these reasons, storage of identified, valuable, and minute specimens in a wet collection has its pros and cons as alternative to storage on microscope slides.

In entomological collections, insect genitalia are also glued with a water-soluble glue on the tip of elongate-triangular strips of cardboard pinned with the insect's body. Genitalia are dissolved with water for investigation and studied in glycerol as a temporary microscope slide. Also, the genitalia may be mounted in different orientations for investigation (Jäger, pers. com.). From a curatorial point of view, this procedure may represent at least a longer-lasting and safer solution than mounting the genitalia on plastic strips or storage in plastic tubes with a cork or silicone rubber stopper. However, genitalia glued to a strip of cardboard are more vulnerable to mechanical damage if the specimen is not handled carefully enough.

3.12 Digitization of microscope slides

Mass digitization of natural history collections at an industrial scale is strongly advocated by some authors (e.g., Blagoderov *et al.* 2012). This includes scanning horizontally stored slides with the SatScan® system (Blagoderov *et al.* 2012), which would result in images of labels and the outline of specimens at best. A more realistic and more sophisticated approach to digitization was suggested by Jersabek *et al.* (2003a, b), Jersabek (2005), and Ang *et al.* (2013). These authors encourage digitization only "if they (i) enhance taxonomic output and quality, (ii) are feasible, and (iii) have favorable cost-benefit ratios" (Ang *et al.* 2013, p. 637). Consequently, digitization is proposed on user demand thus "integrating science and digitization" (Ang *et al.* 2013, p. 638). There are major drawbacks in digitization of microscope slides concerning both general considerations and technical limitations:

(1) Digitization does not stop deterioration of the original microscope slides in any way but takes staff time and expertise, which is then lacking for restoration efforts (see also Ang *et al.* 2013).

(2) Slides have to be cleaned and possibly restored in a time-consuming procedure (comp. Tab. 13) before any images can be taken. It would be of limited use to digitize deteriorating slides with cavities, cracks, and crystals in the mounting medium or even on the specimen. Publishing such images in a publicly available database may be counter-productive, because this would openly demonstrate lack of care by a museum.

(3) It requires considerable time and expert knowledge to take meaningful photographs of exactly those characters, which are taxonomically valuable for a database of, e.g., type and rare specimens (Jersabek *et al.* 2003a, b; Jersabek 2005; Ang *et al.* 2013). Also, each slide would have to be digitized individually with a motorized microscope, especially if DIC or phase contrast are required (see also topics 7 and 8). Such efforts will be even more problematic if a collection is comprehensive and contains numerous different supraordinated taxa, for which experts are lacking at a given museum.

(4) Digitization of a microscope slide cannot replace the physical specimen, because in the future additional morphological characters may become important, which were not documented by images, e.g., because the specimen was not orientated in a favorable position. A physical specimen may be re-mounted in a different position but not an image. Also, future new techniques of investigation may be applied to a physical specimen but not to a digital image, so keeping only images would actually limit future research. On the other hand if specimens are deteriorating rapidly, stacks of images may be all that remains from a specimen.

(5) Bulk scanning of microscope slides may be used for the presentation of collections to the general public, but laymen are certainly more attracted by beautiful or interesting images rather than by masses of microscope slide images without any meaningful information to them. However, it does take considerable time and effort to take interesting images and to provide adequate information about them (see topic 3).

(6) For scientific purposes, stained histological sections and mounts of spicules of Porifera seem to represent the most promising applications of bulk slide scanning currently, because stains fade over time (see chapter 3.7.1 General aspects of mounting media) and may be "preserved" in digital images if these are taken shortly after the production of the histological slide. Also, the digitized sections may be used for three-dimensional reconstruction of specimens or their organs. Such reconstructions would become possible also for Porifera spicules.

(7) Technical limitations are inherent in current (semi)automatic slide scanners originally designed mainly for the digitization of medical microscope slides yielding bright field and fluorescence imaging. Numerous images of a microscope slide are taken at different focal levels, extended focus images created, and these stitched together into one large image of several gigabytes. Differential interference contrast and phase contrast imaging as well as use of oil immersion objectives are currently not possible with any of the scanners on the market (see also below). The highest resolution with a commercial slide scanner is currently reachable with a Zeiss Planapo 40x/0.95 corr. in a Zeiss Axio Scan.Z1; however, the correction of the thickness of the coverslip has to be done individually and manually for each slide, so batch processing is not possible with this objective. One of the more flexible scanners on the market, Zeiss Axio Scan.Z1, offers at least custom-made adaptation to various sizes and thicknesses of microscope slides beyond the standard glass slide as well as ring aperture contrast. However, a number of slides at the Museum für Naturkunde Berlin possess lateral stripes of glass or cardboard, which increase the thickness of a slide to up to 4.6 mm. Such slides certainly cannot be scanned with a currently available digital scanner and have to be handled individually with an upright microscope. Although 100–200 slides may be digitized in one batch overnight, cleaning and calibration of every single slide before digitization is manual and requires time.

(8) Technical limitations are also inherent in current software programs for stacking images to an extended focus image if it involves Nomarski differential interference contrast images. In an upright microscope, the prism in the condenser creates two light beams, which pass the specimen and are re-united by the prism above the objective (Lang 1968, fig. 8; Bradbury & Evennett 1996, fig. 7.7). These two light beams are diffracted in a slightly different way by the specimen depending on the density of the specimen at that point and at the focal level, where each light beam hits the specimen. Consequently, the light beams are no longer parallel anymore and have also slightly moved laterally. Therefore, the images from a stack of images are taken along the slightly oblique light beams, in some way similar to the beams in a Grenough stereo microscope, and structures do not appear completely aligned anymore. Stacking of more than a few DIC images will often lead to artifacts in an extended focus image, especially for spines or at the margin of a specimen. Also, stitching of images does not seem to work (Betz pers. com.). Consequently, stacks of DIC images may be taken as documentary records, but images may better be stored individually.

In summary, bulk scanning does not really represent an alternative to slides, neither for outreach activities nor for most of scientific research. On the contrary, even digitization of certain slides by an expert in the highest quality possible may just be complementary to physical microscope slides in a few specific cases only (Jersabek *et al.* 2003a, b; Jersabek 2005; Ang *et al.* 2013).

3.13 Study of specimens and documentation

3.13.1 Microscope equipment

Objectives. The higher the numerical aperture of an objective, the more the quality of the image depends on the thickness of the coverslip, for which the objective has been corrected by the manufacturer, because the image will otherwise appear blurred due to spherical aberration (see also chapter 3.6 Coverslips; Adam & Czihak 1964, p. 78; Zölffel 2011). In oil immersion objectives, the oil offers the same refractive index as the glass, so the working distance of the objective primarily limits the focal range that can be studied with the lens. Dry objectives with a high numerical aperture usually possess a correcting collar for adjusting to the correct thickness of the coverslip-mounting medium complex; the collar was introduced by Andrew Ross back in 1837 (Adam & Czihak 1964, p. 78; White 1974; Böck 1989). In older microscopes with a horseshoe-shaped stand, differences of coverslip thickness can be compensated for by a change in the mechanical tube length of the microscope at the draw tube or by a special Jackson tube length corrector first described by Bracey (Bracey 1931; Sartory 1949; Wagstaffe & Fidler 1955, p. 195; Spinell & Loveland 1960, pp. 78–79; Adam & Czihak 1964, p. 78). Special objectives with a larger working distance allow the user to study thick specimens or inverted glass slides. However, the numerical aperture and, therefore, the resolution is considerably lower in such lenses (e.g., Zeiss LD Plan-Neofluar 63x/0,75 Korr, working distance 1.7 mm with coverslip 0.75 mm thick, compensation of coverslip thickness 0–1.5 mm) than in an objective with a "normal" working distance (e.g., Zeiss Plan-Neofluar 63x/0.95 Korr, working distance 0.12 mm with coverslip 0.17 mm thick, compensation of coverslip thickness 0.13–0.21 mm). An additional magnification module above the objective facilitates concentration of the microscopic observation on important details without being distracted by information from surrounding tissue, e.g., with the help of a Zeiss Optovar 1.25x / 1.6x / 2.0x or an Olympus module U-CA 1.25x / 1.6x / 2.0x. The magnification module is also recommended for dry 63x objectives in order to reach an increased total magnification. The highest secondary magnification lens is not recommended for photography, because the magnification reached does not provide a higher resolution and is often referred to as "empty magnification" (Piston 1998). Total magnification should not exceed 500–1,000x the numerical aperture of the objective (Gill 2013, p. 322). Alternatively, oculars with 16x magnification may be used for observation instead of the regular 10x oculars, but limitations concerning total magnification apply here as well.

Contrast. Macerated arthropod specimens are often stained in order to make delicate structures visible in transmitted light bright field microscopy (comp. chapters 3.1.4 Staining and 3.10.7 Overly macerated specimens). Entire specimens of meiofauna are commonly studied with differential interference contrast in order to observe morphological details differing with minimal contrast. Phase contrast microscopy may represent an alternative for very flat specimens showing small halos with this system, especially if objectives of highest magnifications are used (Noyes 1982; Disney 1989; Noyes & Polaszek 1989; Neuhaus 1993, figs 6 and 11; Lillo *et al.* 2010). More

recently, tardigrade and mite autofluorescing cuticular structures have been investigated with the help of epifluorescence using UV light (330–485 nm) and confocal laser scanning microscopy using blue light (405 nm), respectively (Chetverikov 2012; Perry *et al.* 2015). Whereas gum-chloral media resulted in a good image quality of internal genitalia and external morphological characters of mites, staining a specimens with iodine or mounting in media containing poly(vinyl alcohol) lead to a severe reduction of the autofluorescent signal (Chetverikov 2012). This author also reported photo bleaching by the laser within 0.5–3 minutes depending on the species. Autofluorescence decreased severely within one year after mounting, and especially internal details became less visible (Chetverikov 2012). Nevertheless, Chetverikov (2016a, p. 378) suggested "that CLSM can be a very useful method for studying old, opaque, darkened or poorly-cleared specimens which would otherwise be difficult to remount".

A home-made combination of a Carl Zeiss Jena interphako tube with a Leitz Dialux stand and interference contrast condenser allows simultaneous, continuously modulated interference and phase contrast observations with considerably fewer optical artifacts than the individual contrast techniques alone (Piper & Piper 2014). Phase contrast, Zernike phase contrast, DIC, and Hoffmann modulation contrast images have now also been emulated by software from transmitted light bright field images in a confocal microscope equipped with a transmitted light detector (Cody *et al.* 2005). This quantitative phase-amplitude microscopy technique may also help in getting useful images from overly macerated specimens or from mounting media that mask certain characters like Canada balsam. Since the technique is based on stacks of transmitted light bright field images, a regular bright field microscope plus the stand-alone software are sufficient to get the software-generated, contrast-enhanced images mentioned before (Barone-Nugent *et al.* 2002).

Insect genitalia are sometimes mounted on a small plastic strip attached to the needle with the remaining specimen and the labels (see chapter 3.4 Slides). The genitalia can generally be observed with a microscope if the plastic strip is placed on a standard glass slide. Investigations using differential interference contrast will probably lead to interference with the plastic strip. If this cannot be tolerated, a microscope designed for studies including plastic materials can be used, e.g., an upright microscope Zeiss AxioScope equipped with PlasDIC. This technique uses a slit diaphragm in the condenser to generate two parallel light beams that are polarized and re-united by a polarizer and a prism above the objective after having passed through the specimen and the objective (Wehner 2003; Danz *et al.* 2004); consequently, no polarized light interferes with the plastic strip.

Condenser. The optimal image quality of an objective is only obtained if Köhler illumination is ensured (Gill 2013, pp. 309313, fig. 19.2). Objectives with a numerical aperture of nD > 0.95 benefit greatly if the common dry condenser with a numerical aperture of nD = 0.9 is replaced by an oil immersion condenser with an aperture of nD = 1.4. This procedure requires oil immersion of the microscope slide from both sides, but the extra effort will be worth it for very delicate structures. Slides thicker than standard glass slides may not allow the user to place the dry condenser with a numerical aperture of nD = 0.9 in the correct position for Köhler illumination, so observation with objectives of a high numerical aperture will suffer. This can be partly overcome by using a condenser with a larger working distance and a lower numerical aperture like nD = 0.63, but this will result in poorer resolution of the image of the objective as well (see previous paragraph). In any case, a compromise must be chosen for observation of thicker microscope slides. Observation of a double-coverslip mount from its reverse side may lead to the problem that the condenser cannot be moved close enough to the slide, because the slide frame may be too thick, and the microscope stage limits the movement. In many Carl Zeiss (west) microscopes, the screw limiting the movement of the condenser can be removed.

Heavily sclerotized chitinized arthropod cuticle becomes significantly more transparent if illuminated with red or near-infrared light; "the general principle is that using a filter of a color complementary to that of the object will enhance the contrast, whilst using a filter of the same, or a similar color, will diminish the contrast of the object" (Bradbury & Evenett 1996, pp. 28, 29). Study of diatoms in mounting media with a low refractive index can still be facilitated by using blue LED or better UV light in combination with image-processing methods like stacking, averaging, and background-subtraction (Höbel & Sterrenburg 2011). However, great care should be taken to expose specimens and mounting medium to UV light for as little time as possible, because the latter may be seriously damaged by the radiation (Höbel 2016). It seems that Caedax, Canada balsam, and Naphrax are more resistant to UV damage than the unknown medium shown under the previous link (Höbel pers. com.).

3.13.2 Optical surfaces, immersion oil

General remarks. The most basic prerequisite for good quality observations of microscope slides is that both the objective and the slide show a clean surface not contaminated with dust, glass particles abraded from specimen slides, flakes of the microscopist's skin, fibers from clothing, pollen, fingerprints, grease, remnants of immersion oil, and the like. Consequently, a microscope not in use should always be covered with its non-conductive (!) plastic dust cover supplied by the manufacturer, which unlike a regular plastic bag will not attract dust (Duke 2004; Gill 2013; Michalski pers. com.). Also, microscopes should always be located in a dry environment, because fungi may develop on the optical surfaces under humid conditions feeding on the minerals of the lens coating and dirt and etching the glass by their production of acidic, alkaline, and chelating agents (Kerner-Gang 1977; Drewello & Weissmann 1997; Duke 2004; Cordero 2013). The fungi may be removable, but often the lens may be spoiled because of the corroded surface (Drewello & Weissmann 1997; Cordero 2013). In a humid environment, "air conditioning or installing an infrared lamp above the microscope (minimum distance 150 cm or 5 feet)" may help to avoid fungal growth; also, "Carl Zeiss microscope optics are impregnated with an improved antifungus agent (manufacturer: Bayer)" (Zölffel 2011; Rottenfusser, R., Wilson, E.E., Davidson, M.W. Carl Zeiss Microscopy Online Campus: Microscope Cleaning and Maintenance. http://zeiss-campus.magnet.fsu.edu/articles/basics/care.html, accessed 19 April 2016). A list of microbiozides for the protection of glass potentially also used in microscopes was published by Drewello & Weissmann (1997). If air conditioning is available, it must run 24 hours a day in order to avoid an increase of humidity, and optical parts may be stored in a sealed container with silica gel or in a heated container (Cordero 2013).

Cleaning. Nikon recommends cleaning optical surfaces with aqueous fluids like the window cleaner Sparkle™ or blue liquid Windex™ and removing residuals with xylene or mixtures of ether and alcohol (Larson 2001). According to recommendations by Zeiss, slides and optical surfaces may be initially cleaned by blowing loose particles away with a rubber dust blower (= bellows ball) and subsequently by wiping with a wetted and repeatedly folded Whatman lens cleaning tissue 105, which is chemically pure cotton and without any additives or silicone (Zölffel 2011). The most convincing and cheap tool for cleaning glass surfaces in the senior author's experience is absorbent purified cotton wool wrapped around a thin stick (Zölffel 2011). Direct contact of cotton wool with fingers must be avoided in order to prevent contamination with sweat and body fat. Therefore, the cotton bud at the tip of the stick is best rolled in the plastic storage bag of the cotton wool. It is prerequisite to use only medical quality DAB 6 (= Deutsches Arzneibuch 6.0, German Pharmacopoeia) absorbent purified cotton wool, which consists of 100% cotton, is highly absorbent, fluff-free, and does not contain any chemical additives (Anonymous 2016; Bracher et al. 2016a, b; Zölffel 2011). Medical quality absorbent purified cotton wool is defined in various pharmacopoeias such as those of Europe and the United States. Any other cotton wool is impregnated with chemical substances or includes synthetic fibers like viscose (= purified rayon) (Anonymous 2016; Bracher et al. 2016 a, b; Rottenfusser *et al.*: see above). In Germany, Zeiss recommends ophthalmic cotton wool (= Augenwatte, absorbent purified cotton wool) by Kerma Verbandstoff GmbH (Tab. 12). Alternatively, absorbent re-usable polyester swabs (ITW Texwipe CleanTips®) work well on the optical surfaces of a microscope (Zölffel 2011). Facial tissues and microfiber cloth must never be used for cleaning optical surfaces of a microscope because of their abrasive properties; for the same reason, any wiping of optical surfaces must be done with a wetted lens cleaning tissue or absorbent purified cotton wool (Rottenfusser *et al.*: see above). Any wiping of an optical surface should start in the center and rotate in a corkscrew motion to the outside in order not to redistribute dirt on an already cleaned area again; for each sweep, a new piece of lens cleaning tissue or absorbent purified cotton wool should be used (Duke 2004; Zölffel 2011; Rottenfusser *et al.*: see above).

The rubber dust blower should not be substituted by a compressed gas duster (= dust-off spray, compressed gas duster), because the aerosol propellant, extremely cold air, or re-liquefied gas may be deposited on the optical surface to be cleaned and may damage it (Duke 2004; Rottenfusser *et al.*: see above). The optical surface may be carefully fogged with one's breath (avoid contamination with saliva), or the absorbent purified cotton wool may be wetted with a small amount of distilled water or alternatively with a volatile organic solvent such as petroleum ether or a mixture of analytical grade 85% *n*-hexane and 15% isopropyl alcohol, which evaporates before possibly dissolving the lens cement or the coverslip seal (Tab. 10; Zölffel 2011; Rottenfusser *et al.*: see previous paragraph). The isopropyl alcohol extends the retention time on the optical surface (Rottenfusser *et al.*: see above). Duke (2004) suggests carbon tetrachloride, diethyl ether, freon ff, heptane, methylene chloride, naphtha, toluene,

turpentine, and xylene for cleaning Cargille immersion oil, but these are considerably more hazardous to humans or the environment than hexane and ispropyl alcohol. Many organic solvents may leave residues on the optical surface (diethyl ether, ethanol, toluene, xylene) or dissolve the cement of the front objective lens (acetone), the plastic and rubber parts of the oculars (acetone, chloroform, methylene chloride), or the paint on the microscope (acetone, xylene) (Duke 2004, p. 44; Rottenfusser *et al.*: see above). The lens cement of objectives from older Carl Zeiss microscopes, often Canada balsam, is sensitive to acetone and ethanol, whereas the lens cement from more recent microscopes, usually polyacrylic resins, is not (Zölffel 2011; Rottenfusser *et al.*: see above). Care should be taken, when organic solvents are used, not to come into contact with the coverslip seal or any mounting medium, because the solvent may react with these substances. In contrast to Larson (2001) and Duke (2004), Zeiss does not favor household cleaning agents at all, because they often contain ammonia or acids, both of which may dissolve the antireflection coatings if applied repeatedly (Zölffel 2011).

Immersion oil. Different immersion oils have been used during the past century, several with severe drawbacks for the slides or the health of the observer. Immersion oils may generally lead to degradation of the coverslip seal, become tacky, or thicken (Hooper 1986b). Cedarwood oil was used in the 19th and 20th century as an immersion oil (Romeis 1948; Böck 1989). However, this natural oil dried over time and finally solidified (Adam & Czihak 1964, p. 170), so objectives and coverslips had to be cleaned immediately after usage. Also, methyl benzoate seems to have been applied for the same purpose, but this may dissolve coverslip seals and mounting media quickly (von Knorre pers. com.). Older literature also suggests anisole, paraffin oil plus 1-bromonaphthalene, and olive oils plus 1-bromonaphthalene as immersion oils (Adam & Czihak 1964, p. 171). Anisole evaporates quickly (Tabs 4, 10) and attacks the cement of front lenses (Rottenfusser *et al.*: see above), and all four media are hazardous, so these media certainly do not qualify for use today. Immersion oils by Cargille, Carolina Biological Supply Company, Crown, Fisher, Harelco, Nikon, and Zeiss in Europe, North America, and Japan consisted until about the end of the 1970s of polychlorinated biphenyls (PCBs), but this fact was not indicated on the container (Bennett & Albro 1973; Lee *et al.* 1999). The PCBs were replaced by mixtures containing chlorinated paraffins, which also constituted a health hazard (Sacher 1984: mixture of a dialkyl phthalate, a butyl benzyl phthalate, and chlorinated paraffin, US patent 4,465,621 for Cargille Laboratories; Tanaka 1988: mixture of polybutadiene with one or more components of chlorinated paraffins, polybutene, carboxylic acid esters, liquid paraffins, saturated aliphatic alcohols, and alicyclic alcohols, US patent 4,789,490 for Idemitsu Petrochemical; Carl Zeiss Immersion oil 518 C, composition cited in: Weippert 1998: mixture of chlorinated paraffins and di-*n*-butyl phthalate, US patent 5,817,256 for Carl Zeiss and MSDS from 13.X.1995; Lee *et al.* 1999). Leica, and possibly other companies, substituted the chlorinated paraffins with epoxy resin components causing contact dermatitis (e.g., Lee *et al.* 1999). Leica withdrew this immersion oil in 1997. The main ingredients of the current immersion oils Zeiss Immersol™ 518 F and Immersol™ 518 N are adipic acid-di(8-methyl-tricyclo(5,2,1,0,2,6)decane)ester and benzylbenzoate and of Immersol™ M octahydro-4,7-methano-1H-indenmethanol and oxidipropyl dibenzoate according to the MSDS (Available from: https://www.micro-shop.zeiss.com, accessed 30 May 2017). Crystals form in an older formula of Leica Immersion Oil, item No. 11 513 859, and of Zeiss Immersol™ 518 F. Both oils nevertheless offer high quality immersion observation, and crystals in the oil can be dissolved again by heating the bottle with the oil but without the plastic lid in a microwave for about 10–20 seconds or in a water bath at 40–50°C. These oils stem from the same source. More recent formulae of Leica Immersion Oil and Zeiss Immersol™ 518 F do not form any crystals over time.

Immersion oil should be removed from an immersion oil objective daily, because some oils tend to harden or form crystals over time and may even enter the objective, especially in inverted microscopes (Larson 2001; Rottenfusser *et al.*: see above). Different immersion oils or even different batches of the same oil should not be mixed in order to prevent potential crystallization problems (Duke 2004; Zölffel 2011). It remains open how hazardous immersion oils may turn out to be in the future. Therefore, contact of skin with immersion oil should always be minimized, and old immersion oil should be disposed of according to hazard regulations.

3.13.3 Documentation

Generally, specimens studied for taxonomic purposes should be documented as extensively as possible, e.g., by light micrographs, SEM or microtomographic images, and illustrations, also because the slides of the original type

material may deteriorate and the published images may be all that remains from a collection (Amrine & Manson 1996; Lillo *et al.* 2010). In addition, images and other documentation taken during a life-long career in taxonomy are often lost for the scientific community with the retirement of a scientist. This may be partly overcome if data is stored at one of the larger natural history museums (e.g., physical illustrations on paper) or in a database designated for morphological data such as MorphBank ALA (http://www.morphbank.ala.org.au, accessed 09 November 2015; see also Fisher & Dowling 2010) and MorphDBase (= Morphological Description Data Base; https://www.morphdbase.de/, accessed 09 November 2015). Alternatively, image data may be stored in a specimen data base of a natural history museum.

Taxonomic studies usually include drawings of a specimen. The easiest way of getting such an illustration is certainly using a drawing tube (= camera lucida) attached to a microscope and outlining the interesting structures with pencil. The term "drawing tube" must not be mixed with the "draw tube" of the older microscopes with a horseshoe-shaped stand; in the latter, the tube was used to compensate the varying thickness of the coverslips by adjusting the mechanical tube length (see previous chapter; Wagstaffe & Fidler 1955, p. 199; Spinell & Loveland 1960; Adam & Czihak 1964). Detailed instructions about how to proceed with a drawing tube for taxonomic illustrations were provided by Lillo *et al.* (2010). The draft taken with the drawing tube may be subsequently either drawn in ink on an overlaying sheet of semitransparent paper or digitized with a flatbed scanner, imported into Adobe Illustrator, and drawn digitally with the help of this program and a Wacom Intuos digitizer board (Coleman 2003). Coleman (2006) later suggested macerating specimens like small crustaceans, taking serial stacks of images of the relevant structures thus replacing the pencil draft illustration, importing the images into different layers in Adobe Illustrator, and drawing digital lines on top of the images in a separate layer. His latest improvement refers to drawing setae, spines, and complex setae in a more sophisticated way with the software Adobe Illustrator (Coleman 2009; Fisher & Dowling 2010). The drawing tubes designed for Zeiss Axioskop and Axioplan as well as for the Leica DMR belong to the best corrected tubes ever produced with a possibility for post-magnification and with the least amount of distortion. This is very important if a specimen has to be moved on the stage for complete illustration. These tubes are no longer manufactured and do not fit to the current Zeiss AxioImager, Axiovert, and AxioScope series. A more recent and more simple drawing tube by Leica does not allow post-magnification and reveals more distortion in the periphery. Olympus offers adaptation of a 2x objective to the drawing tube in order to draw more details in the illustrations.

If no drawing tube is available, proportions may be estimated with the help of a grid plate in the correct ocular plane and transferred with pencil on a sheet of paper. More recent digital solutions include a combination of a digital camera Zeiss Axiocam ERc 5s mounted on a microscope, the free software Zeiss Labscope, and an Apple iPad. The option "drawing tube" in the software allows to digitally draw on the iPad (more information Available from: https://www.zeiss.com/microscopy/int/products/microscope-software/labscope-for-windows.html, accessed 30 May 2017). Another approach suggested a digital video camera mounted on a microscope, the free VLC media player used in overlay mode to establish the live microscope image as a background for the drawing, an open source drawing software such as GIMP for raster graphics or InkScape for vector graphics, and a digitizer board (Sidorchuk & Vorontsov 2014). Since the previous solution did not work with the operational system at his institution, Chetverikov (2016b) projected the live image from the microscope with a digital projector on a digitizer board for drawing.

4. Concluding remarks

4.1 Mounting medium and coverslip seal: durability *versus* reversibility

Each mounting medium has its advantages and disadvantages concerning handling, microscope investigation, long-time conservation properties, and reversibility of the mount (Tabs 6, 13). The latter two criteria seem to be the most important to evaluate mounting media of microscope slides deposited in museum collections. Although mounting media and coverslip seals reveal different chemical ingredients, all of them except glycerol are composed of at least one polymer and at least one solvent. From conservation studies, it seems unlikely that a polymer can combine durability with reversibility. Polymers are linear, branched, or cross-linked. Only linear, branched, and slightly cross-linked polymers are expected to be soluble in hydrocarbons and, therefore, will lead to reversible mounts (Baker 1995, pp. 306–307; Mills & White 1999, p. 130; Horie 2011, p. 105). A polymer will become more

durable if extensive cross-linking occurs within the polymer. However, it must be kept in mind that a polymer cannot be dissolved by solvents or mild heat again once the polymer has undergone extensive cross-linking; such a polymer has to be considered as irreversible (Mills & White 1999, p. 130; Horie 2011, pp. 105–106). Deterioration of resins with age certainly includes a complex pattern of chemical reactions including cleavage, "cross-linking, inter- and intramolecular cyclization reactions and defunctionalization by loss of acidic groups", e.g., "in Manila copal and sandarac, the degree of cross-linking increases with age, but it is counterbalanced by the decrease in the molecular mass of polymeric material" (Scalarone *et al.* 2003b, p. 615).

Deterioration of polymers is mainly supposed to take place via chemical processes like oxidation leading to complex reaction patterns including cleavage of chains, isomerization, and cross-linking (e.g., Rie *et al.* 1988b; McNeill 1992; Scalarone *et al.* 2002, 2003a, 2003b, 2005; Colombini *et al.* 2003; Dietemann *et al.* 2009; Horie 2011, pp. 38–43; Coelho *et al.* 2012). For picture varnishes, it is well known that the degradation of the polymer will lead to yellowing, cracking, change in solubility, and increased fluorescence (Feller 1971; Rie 1988b; Dietemann *et al.* 2009). Physical deterioration of polymers mainly used as picture varnishes results in cracking, shrinkage on drying or in loss of a plasticizer, flowing, and absorption of dirt (Horie 2011, pp. 43–44). For microscope slides, it has additionally been found that crystals and cavities may develop, ingredients may segregate, and coverslips may detach (Tabs 5, 6). Also, fixing, dehydrating, macerating, and clearing agents from previous steps of preparation may be dragged along into the mounting medium and cause degradation over time, e.g., crystals or black granules may form on a slide if the specimen has been fixed with a mercury compound (Gray 1954, p. 255). The situation becomes more complex by the fact that usually not all slides prepared at a given time in the same way are impacted equally (Spence 1939; Bink 1979; Eastop 1985; Hooper 1986b; Noyes & Polaszek 1989; Upton 1993). Based on our unpublished results (Julia Hidde, Thomas Schmid, Jens Riedel, Birger Neuhaus), we suspect that at least some synthetic resins applied for microscope slides suffer more from obvious physical changes like crystallization, formation of cavities, and segregation of components than from chemical changes.

No single reason can be held responsible for the deterioration of slides, numerous arguments have been put forward (comp. discussion above and Tab. 7; Upton 1993). However, any organic polymer will deteriorate over time because of exposure to oxygen, light, temperature changes, humidity, and volatile molecules from the environment (deterioration of cabinet, pollution, building, etc.) as well as because of the chemical composition of the polymer including impurities from manufacture (McNeill 1992; Davison 2003, p. 208; Dietemann *et al.* 2009). Deterioration occurs mainly via autoxidation by free radicals and via ionic reactions often mediated by acids and other catalysts (Mills & White 1999, p. 160). Acids may originate from macerating agents incompletely removed before mounting and from acids included in the mounting medium on purpose (see chapters 3.1.2 Chemical maceration and 3.7 Mounting media). Gum-chloral media may be more prone to deterioration than other mounting media based on natural resins, because the glycoproteins and the polysaccharide of the gum arabic seem to be less stable than the chemical ingredients of other natural resins (comp. Tab. 5). Environmental factors, and here particularly UV light, are thought to initiate autocatalytic autoxidation processes as oxidative radical chain reactions, which are well known for gum dammar and are very likely for other natural resins with abundant carbonyl groups (Rie 1988b). Consequently, compatibility and ageing tests have been suggested to establish in natural resins with hindered amine light stabilizers of high molecular weight, which do not evaporate readily (Rie 1988a). Exposure to sunlight during harvesting of gum mastic has been assumed to start autoxidative processes, and autoxidation took place to a considerable degree both in resin stored in the dark and exposed to light (Dietemann *et al.* 2001). However, a later study indicates (1) that autoxidation is not started by light but presumably by atmospheric compounds like SO_2 and NO_2 and (2) that the polymer fraction of the resin harvested in daylight, which is missing in resin harvested in the dark, acts as a natural radical scavenger (Dietemann *et al.* 2009). This also means that the light stabilizers suggested by Rie (1988a) will probably not stop autoxidative processes but merely slow them down.

The complete list of chemical ingredients is unknown for any mounting medium and coverslip seal. The chemical composition also varies with manufacturer and time. For these reasons, the Committee on Histological Mounting Media suggested that the manufacturer should provide detailed information of the chemical and physical properties of their mounting media (Lillie *et al.* 1953, p. 72). Most mounting media and coverslip seals listed in Tables 5 and 8 do not seem to have been checked for reversibility by restorative activities. These aspects do not currently allow a fully objective evaluation of mounting media and coverslip seals. However, some conclusions can still be drawn from the data gathered together in this paper:

(1) For some coverslip seals like Araldite, asphaltum, Murrayite, nail varnish, and silicone rubber (Tab. 9) as well as for a range of mounting media like Aquatex®, Bio-Plastic®, Caedax, Clarite, Clarite X, CMCP-9, CMCP-10, DePeX/ DPX, dimethyl hydantoin formaledehyde (DMHF), Entellan® new, Eukitt™, Fluoromount G™, glycerol-gelatin (Kaiser's; Zirkle's acetocarmin), all gum-chloral media, Permount™, Polylite®, polyvinyl lactophenol, Polyviol, Technovit® 7100, Venetian turpentine medium after Wilson, and Water-glass-glycerol, **deterioration processes** within a few months or years after mounting are well documented, namely frequent formation of cavities and crystals and segregation of components (Tab. 6). Restoration by soaking is not known for most of these mounting media or not totally successful for CMC-10 and Permount™ (see chapter 3.10 Restoration procedures). None of the media listed above can be recommended for mounting biological specimens at all, because extra curation time is required for monitoring slides and for restoration efforts, which inherently bear the risk of damaging or losing a specimen. Also, restoration has to be executed before crystals destroy a specimen.

(2) For many coverslip seals like Corseal and mounting media like cellulose caprate, C-M Medium, CMC-9, CMC-10, Loctite® UV 357, Malinol, and Visikol™, little or no information is available about their principal ingredients, longevity and reversibility (see also chapter 2. Material and methods for additional media; Tabs 6, 9). In the case of Loctite® UV 357, it turns out that even the manufacturer cannot provide information about major ingredients 25 years after its last documented application in medical histology. Such media should be avoided until more rigorous tests have proven their suitability for long-time storage in museum collections.

(3) Macerating agents in media like CMC(P)-9, CMC(P)-10, dimethyl hydantoin formaldehyde, glycerol-gelatin (Zirkle's acetocarmin), all gum-chloral media, lactophenol gel, polyvinyl lactophenol, Polyviol, Venetian turpentine medium after Wilson, and Visikol™ continue to destroy a specimen chemically. Therefore, these media do not represent recommendable mounting media for the long-time storage of valuable biological specimens.

(4) With regard to **longevity**, a medium containing a plasticizer such as Araldite, Caedax, Caedax A, Caedax 547, cellulose caprate, Clarite, Clarite X, DPX/DePeX, nail varnish, Naphrax™, and Rhenohistol cannot be regarded as suitable for microscope slides in museum collections, because plasticizers are known to migrate out of polymers over time (Tabs 5, 7, 10).

(5) Polymer chains of media like epoxy resins and polyesters may cross-link with each other and for this reason cannot be dissolved by solvents again, so mounts with these media are **not reversible** (Witte 1983; Horie 2011, p. 17). Consequently, such media can be recommended as mounting media and coverslip seals only with some reservation. Reversibility of restoration activities has been recognized as an important prerequisite in art conservation for a long time (Shashoua 2008; Horie 1983, 2011) but neglected in microscope slide preservation.

(6) There is no optimal medium covering all aspects, but **neutral Canada balsam** with a reported life time of over 150 years and **Euparal** with a reported life time of at least 50 years seem to unite most good qualities and especially the long-time stability (Tabs 6, 14; Brown 1997). Some of the drawbacks of these media seem to be related to unfavorable combinations with clearing agents like phenol (Tab. 6). For histological sections stained with acidic stains, an alternative to Canada balsam does not seem to exist in terms of long-time storage. For alkaline stains, only Euparal seems to be recommendable because of the acidity of Canada balsam. The long-time stability of neutral Canada balsam and Euparal may not come as a surprise, because both media consist of natural resins like amber, and the latter survives millions of years under proper conditions.

(7) For total mounts where Canada balsam and Euparal would mask characters or make specimens brittle because of the dehydration required, the **double-coverslip glycerol mount with Canada balsam or Euparal** and the **glycerol-paraffin mount** with a proper coverslip seal do not form cavities and crystals and can be re-mounted with less effort than other mounting media and so fulfill the criterion of reversibility. Therefore, these techniques seem to represent a good option for total mounts despite the need of horizontal storage (generally recommended for microscope slide anyway) and of more regular control (Tabs 6, 9, 14).

(8) The evaluation of mounting media for museum collections in this paper pays little attention to the refractive index of the media, because different preparations of different taxa benefit from different refractive indices of the mounting media. However, phase contrast, differential interference contrast, and software emulating different contrast techniques may partly overcome this problem (see chapter 3.12.3 Microscope equipment).

(9) Glyceel and possibly **Glyptal** seem to represent a permanent-elastic seal for the coverslip in order to stabilize the mount mechanically and to limit exchange of water between mount and environment. Alternatively, the **double-coverslip technique with Canada balsam or Euparal** may be applied (Tabs 9, 14).

TABLE 16. Recommendations for microscope slide collections and their study.

Topic	Recommendation
Initial treatment of specimens	fixation in formaldehyde for at least 1 week or in glutardialdehyde for morphological studies, ethanol conservation may be sufficient for studies of cuticle only complete removal of macerating agents by repeated washing and/or neutralization
Storage	collection room at consistent temperature of about 18°C and about 60% relative humidity cabinet protecting efficiently against light and dust metal cabinet with stoved / baked enamel coating based on powdered paint electrostatically applied to the metal surface wooden cabinets: check for volatile acids with the help of Oddy test, improvement of ventilation of collection room, and usage of gas sorbents in the cabinet (activated charcoal, with or without molecular sieve) horizontal storage of slides in order to keep loose labels and coverslips as well as liquid-mounted specimens in position on slide trays made of (anodized) aluminum, steel with stoved / baked enamel coating based on powdered paint, or archive-quality cardboard only one specimen mounted per slide
Collection management	review of slides by profiling every 10–15 years, control of specimens mounted in liquid media every 5 years material given on loan to other scientists may exclusively be mounted in long-lasting or reversible media but not on plastic strips as is or has been common practice in entomological collections detailed documentation of preparation technique for each slide in catalogue / database cleaning of slides with the help of absorbent purified cotton wool (e.g., ophthalmic cotton wool = Augenwatte from Kerma), skewer, and distilled water
Slide	standard glass slide, hydrolytic class 3 soda-lime glass, cleaned, extra white, free of surface defects, inclusions, streaks, and bubbles, with ground edges coverslip No. 1 (0.130–0.160 mm), hydrolytic class 1 borosilicate glass D 263™, extra white, highly transparent, colorless, free of blisters and flaws for double-coverslip mounts (study of specimen also from reverse side): Cobb aluminum slide permanent and clear identification by scratched-in catalogue number on slide (with diamond-tipped or tungsten carbide-tipped engraving scribe, electrical engraving tool) mounting medium: long-lasting (Canada balsam, Euparal) or reversible (double-coverslip glycerol mount with Canada balsam or Euparal; glycerol-paraffin mount) coverslip seal: Glyceel, possibly Glyptal, double-coverslip technique with Canada balsam or Euparal
Label	data in natural language, no codes, no abbreviations archive-quality paper according to DIN/ISO 9706 labels printed with laser printer (heat treatment if necessary) / light- and ethanol-proof pigment ink (e.g., Edding profipen 1800, Faber-Castell ecco pigment, Higgins T-100, Hunt speedball super black ink, Pelikan 17 black, Pelikan 50 special black, Rotring 17 black) label glued with archive-quality, water-removable adhesive (e.g., Klucel G®)
Study of slides	condenser adjusting according to Köhler illumination after each change of objective long-distance lens (LD lens) for study of inverted glass slide additional magnification module above objective or 16x-oculars facilitating concentration of observation on important details without being distracted by information from surrounding tissue oil immersion / multi-immersion objective for highest resolution and immersion condenser (nA = 1.4) clean optical surfaces with rubber blower ball, use absorbent purified cotton wool (e.g., opthalmic cotton = Augenwatte from Kerma) rolled around skewer with distilled water subsequently, apply analytical grade 85% n-hexane and 15% isopropyl alcohol for more resistant dirt documentation of new species by light micrographs and illustrations in original publication as extensively as possible
Description of new species	digital inking of pencil drawing with Adobe Illustrator and digitizer board

4.2 Future studies

Little is known about the long-time conservation properties and reversibility of many media except a few, *inter alia*, because the mounting medium is often mentioned neither on the slide nor in the publication. Also, not all components of the media are known because of proprietary manufacture and changes in recipes over time. The need for a long-lasting and/or reversible mounting medium for microscope slides is without an alternative considering the problems summarized above. However, this requirement leads to a dilemma well expressed by Criado-Fornelio *et al.* (2014, p. 147) "Brown (1997) pointed out that a conservative approach should be envisaged by museum curators, employing always traditional permanent mounting media for preservation. Notwithstanding, such idea should not apply to research institutions. Indeed if no new preservation procedures are developed, the major drawback in current protozoological techniques shall never be evaded." The main problem in our opinion is that scientists suggesting a "better" or "alternative" mounting medium usually do not and cannot base their recommendations on long-time observations, experience is usually limited to a few months, a year, and sometimes several years—this is close to nothing in comparison with the time horizon for the storage of type and voucher material in museum collections. Even a "hundred year permanence" (Thatcher 1987) may not satisfy the needs of museum collections. Therefore, we support the suggestion of the Conservation and Preservation of Natural Science Collections Project of the National Institute for the Conservation of Cultural Property, USA that biologists should cooperate with researchers from materials sciences (Duckworth *et al.* 1993) and concentrate their efforts on understanding the chemical and physical properties of the ingredients of mounting media and coverslip seals as well as their interaction with the glass surfaces and the specimen. A comparison of the aged resins of amber and of those used in art and to preserve mummies centuries and millennia ago (Mills & White 1977, 1999; Daher *et al.* 2010; Edwards 2005; Edwards & Ali 2011) with the ageing of mounting media composed of natural resins may help to understand deterioration of such mounting media. In addition, we strongly encourage scientists to undertake rigorous accelerated ageing studies of a new medium before recommending it as "permanent". As long as such examinations are missing, we completely agree with Brown (1997) who proposed applying only those media that are known to be more durable or reversible (see previous chapter).

Non-destructive methods from materials sciences for the recognition of a mounting medium have not been applied to biological and geological slide preparations except in three cases (Garner & Horie 1984; Valentine-Baars & Kerbey 2014; Schmid *et al.* 2016), but at least some Raman spectra of natural resins and of the mounting medium Entellan® new are Available from: art, archaeological, and forensic sciences (Edwards *et al.* 1996; Daher *et al.* 2010; Yu & Sandercock 2012). In a first step to overcome the disappointing situation for microscope slides, a database of Raman spectra of the known chemical components of mounting media and coverslip seals as well as spectra of freshly made media has been started by our team (Schmid *et al.* 2016). These studies will be continued and complemented by investigations on accelerated ageing of model slides compared with "naturally aged" slides.

4.3 Outlook

There may not be final solutions to many problems concerning the preservation of natural history objects, but pros and cons must be discussed, and arguments have to be balanced against each other in every case. Deterioration of natural history objects is inevitable, but can be slowed down by choice of proper materials and methods or may be accelerated by applying improper materials and methods. The International Code of Zoological Nomenclature requests in its 4th edition that taxonomists store type material of new described species in publicly available collections in order to make such material accessible for future generation of scientists (Schauff 1985; ICZN 1999). However, microscope slides have been and still are made mainly under the aspect of getting a satisfying result for research within a short time. During the past decades it became obvious that many ways of making, preservation, and storage of microscope slides are more prone to deterioration than others, but still little is known about the chemical processes occurring during ageing of the media. Unfortunately, scientists continue to use problematic mounting media irrespective of well-known and extensively documented problems (Tab. 6, Tab. 7 for gum-chloral media; Upton 1993). The sometimes surprizing arguments (Tab. 7) may provide an idea about how long a fundamental change among biologists may take, and only few scientists seem to change their mind about which mounting medium to opt for (e.g., Wahl 1989). Choosing a longer-lasting or a reversible option will decrease the

amount of work and time that has and will have to be devoted to such collections now and in the future (Brown 1997, pp. 10–11) or to quote Upton (1993, p. 273): "Workers who feel that mounting specimens in genuine permanent mountants is too time consuming can be assured that the time required to retrieve and remount their specimens (if indeed this is possible) will far exceed that of using a permanent, resin-type mountant such as Euparal in the first instance." Therefore, transfer of ethical standards and of technology from conservation science is badly needed to understand and hopefully to overcome limitations of current mounting media. In the long run, recipes of mounting media and coverslip seals recommendable for taxonomic purposes should be published and standardized, possibly in a European or even international standard, to ensure standardized media with known components (see also Lillie *et al.* 1953, pp. 71–73; Gill 2013, pp. 263–264). Manufacturers could refer to this standard in their advertising of certified media (Scott 1951). Similar ideas have been discussed and partly implemented for biological stains (Lyon 2000). This author also outlines the problems and drawbacks related to such an endeavour. At some stage, recommendable mounting media and coverslip seals may have to be patented for a foundation and controlled by it in order to keep the production of such a medium as cheap and as reliable as possible for the scientific community interested in taxonomic studies. A similar approach has been undertaken for ground glass stopper jars and for suggestions to stabilize the pH in fluid-preserved natural history collections (Clark 1995; Kotrba & Golbig 2009). It may also be considered to require mounting taxonomically important specimens in proven and certified permanent or reversible media by the International Code of Zoological Nomenclature at some stage.

Ignorance of the problems with mounting media and coverslip seals of microscope slides will pave the highway to hell—for future scientists in need to study slides deposited in museum collections and for museum staff being obliged to make the stored material available to scientists and keep it functioning (Schauff 1985; ICZN 1999). A number of slide collections have already been lost or are close to being lost (Schauff 1985; Sterrenburg 1990; Upton 1993; Brown 1997; Jersabek 2005; Jersabek *et al.* 2010; Lillo *et al.* 2010; Neuhaus pers. obs.). For sure, these few reports represent just the tip of the iceberg, because no natural history museum wants to admit publicly that it is not capable of maintaining its deteriorating collections because of lack of staff and funding. It must be clearly stated, that any kind of biological specimens requires a minimum amount of caretaking over the years in order to just maintain the current status (Sterrenburg 1990; Brown 1997; Cushing 2011). Museums of natural history would be badly advised to cut down scientific and technical staff below this minimum, because this would also drastically limit options for future generations.

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